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DEPARTMENT OF MARINE BIOLOGY
OF
CARNEGIE INSTITUTION OF WASHINGTON

ALFRED G. MAYER, DIRECTOR

PAPERS FROM THE TORTUGAS LABORATORY
OF THE
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I.

THE EFFECTS OF TEMPERATURE UPON TROPICAL MARINE ANIMALS.

BY ALFRED GOLDSBOROUGH MAYER,
Director of Department of Marine Biology of the Carnegie Institution of Washington.

Eight figures and four records.

THE EFFECTS OF TEMPERATURE UPON TROPICAL MARINE ANIMALS.

By Alfred Goldsborough Mayer.

CONCLUSIONS.

Tropical marine animals commonly live within 5° C. of their temperature of maximum activity and within 10° to 15° C. of their upper death temperature. In marine animals of the temperate or arctic regions a considerable range of temperature above or below the normal produces relatively little difference in their activities, but in tropical forms even a few degrees of heat or cold cause a marked depression in movement. In tropical Scyphomedusæ this depression of movement appears to augment about as the square of the change in temperature from that of the optimum. Thus it is more depressant than one would expect were it due to a simple chemical reaction.

As was shown by Harvey (1911), the rate of nerve-conduction in the subumbrella tissue of *Cassiopea* varies as the increment of temperature, increasing in a linear ratio as the temperature is raised.

Experiments conducted at the Murray Islands, Australian Great Barrier Reef, show that those corals which die at temperatures below 36.5° are killed by being buried 11 hours under the mud, but those which resist 37°, and above, are proportionally less sensitive to the smothering effects of mud and may survive being buried for 30 to 40 hours. This suggests that high temperature produces death by causing asphyxiation, the oxygen of the sea-water being insufficient to sustain the increased metabolic activity of the animal.

OBSERVATIONS AND EXPERIMENTS.

The literature upon the effects of temperature on animal life and activities is both extensive and widely scattered, although Davenport and Castle (1895), in their paper in the "Archiv für Entwicklungsmechanik der Organismen," Bd. 2, have given a list of all previous papers treating of experiments upon the maximum temperature which can be endured by aquatic organisms; and Bachmetjew (1899), in the "Zeitschrift für wissenschaftliche Zoologie," Bd. 66, presents a complete résumé of researches upon the subject of the temperature of insects in relation to that of their surroundings. Also a review of works upon the physiological effects of temperature is given by Demoll and Strohl (1909).

Probably no single factor is a more effective barrier to the extensive geographical range of marine animals than is that of temperature. This fact has long been recognized and there are many striking examples of its general truth; for example, Professor Hjort, in Murray and Hjort's "Depths of the Ocean" (1912, p. 444), concludes that the southern limit of northern boreal species of fishes from the sea-bottom everywhere coincides with the isotherm of 10° C. at a depth of 100 meters.

That temperature should be so important a barrier to universal distribution is the more remarkable in view of the large number of observations showing how readily animals may become artificially adapted to survive in unaccustomed temperatures. Thus Davenport and Castle (1895) found that tadpoles reared at about 15° C. go into heat-rigor at 40° to 41° C., but if kept at about 24.5° C. for 4 weeks their heat-rigor temperature is raised to 43° to 43.8° C.

The experiments of Dallinger (1887) are even more remarkable, for he kept *Flagellata* in a warm atmosphere beginning at 15.6° C., gradually raising the temperature for several years until it became 70° C., the colony still surviving and reproducing normally, although in nature the same species were killed at 23° C.

In studying animals in a state of nature, Vernon (1899) found that at Naples the mean death-temperatures of various marine invertebrates ranged from 34° to 42.3° C., but that the death-temperatures of the same species in July and August are from 0.6° to 1.3° C. higher than in March and April, this change being associated with a rise of about 10° in the surface temperature of the Mediterranean. Observations of similar purport were made by King (1903) upon the effects of seasonal temperature on the development of toad eggs.

It is, therefore, somewhat surprising, when we see how readily certain animals may become acclimated in a short time to abnormally high or low temperatures, that whole races of marine organisms are confined within a narrow range of temperature. For example, the rhizostomous scyphomedusæ are restricted to tropical or warm seas, none of them extending into regions beyond the summer isotherm of 16° C. Also, the species of the genus *Cyanea* are equally well confined to cold seas, being bounded in their range from either pole by the summer isotherm of 20°. On the other hand, *Aurellia aurita* is eurythermal, ranging from pole to pole and giving rise to many varieties and local races.

Among the 440 known species of hydromedusæ only one, *Solmundella bitentaculata*, appears to be eurythermal, living at temperatures ranging from 30° to −1° C. and at depths between 1,500 fathoms and the surface, and even among the widely ranging Siphonophoræ, Bigelow (1911) is able to name only one doubtfully eurythermal species among the 90 known forms.

A study of the temperature reactions of the only known eurythermal scyphomedusa, *Aurellia aurita*, is of interest, for it shows that the success of this species in adjusting itself to a wide range of temperature is due to a remarkable ability for acclimatization. Thus, the *Aurellia* at Halifax, Nova Scotia, ceases to pulsate at 29.4° C., at which temperature it is most active at Tortugas, Florida; and conversely, the Tortugas medusæ cease to pulsate at about 7.75° C., while those from Nova Scotia continue to move at a temperature of −1.4° C. with ice floating in the water above them. If frozen solidly into the ice the Tortugas medusæ are killed, but (as was observed by Romanes, 1885), our northern *Aurellia* withstands this treatment without serious injury.

Aurellia aurita is more abundant in cold than in warm seas, however, perhaps on account of the more abundant zoöplankton and food-supply in cold waters; yet it may be due to a better adjustment to environmental temperature in the medusæ of the cold waters, for at Halifax the surface-temperature in summer is about 14° C. and the range in activity of the medusa is then about 15° C., both above and below this temperature, the animals being most active at from 18° to 23° C. On the other hand, at Tortugas the average surface-temperature in summer is about 29° C., being practically identical with that of the animal's greatest activity; but a rise of 8° C. would cause all movement to cease and is generally fatal to the medusæ, while the same species at Tortugas can still pulsate even at a temperature of 21° below that of the ocean in which they live. It appears probable that *Aurellia* is a boreal or arctic animal which has wandered into the tropics and become fairly well acclimated, although living in these warm waters within 9° C. of its death-temperature. Thus, in comparison with their northern relatives, these Aurellias in the tropics are poorly adjusted to their temperature environment, and a change of even 2° or 3° above their normal temperature causes a decided lassitude and loss of rate in pulsation. We see, therefore, that the medusa has become adapted to a tropical environment, but this has been accomplished at the expense of its factor of safe adjustment to temperature changes.

Harvey (1911, p. 34) showed that *Cassiopea*, which is strictly confined to the tropics, can not withstand a higher degree of heat than can *Limulus* at Woods Hole, Massachusetts. Thus, *Cassiopea* is an animal living constantly within 15° C. of its death-point, yet it is not adapted to withstand higher temperatures than the heart of a northern animal (*Limulus*) living at 25° to 30° C. from its death-point.

In general, one finds that tropical marine animals normally live at temperatures much nearer their death-point than do northern forms. For example, the most resistant reef coral, *Siderastræa radians*, which lives at about 28° to 30° C., is killed at 38.5° C., and the West Indian reef Madrepores are killed at about 35.8°. The tropical sea-urchins, *Diadema* (*Centrechinus*) *setosum* and *Toxopneustes* (*Lytechinus*) *variegatus*, and the brittle-star, *Ophioderma appressa*, are killed at 37.4° to 37.7°. In these forms the temperature of the sea-water in summer is only from 7° to 10° C. below the death-temperature. By way of contrast, we may cite the cases of the arctic forms *Cyanea arctica* and *Beroë cucumis*, which are killed at about 27° and 30° C., respectively, and which live in an ocean not warmer than 14° C., thus being at least 13° to 16° C. from their death-points.

This low factor of safety in tropical marine animals may at times become of biological significance. Thus on July 21–22, 1911, at Tortugas, Florida, after several hot, calm days, the shallow water over Bird Key Reef rose to 33° to 38° C. and Dr. L. R. Cary observed that large numbers of *Diadema*, *Octopus*, *Fissurella*, and other mollusks and small fishes were killed in considerable numbers over extensive areas, and corals were injured even when not exposed to the air.

In order that a marine animal may live throughout the year in the shallow or surface waters of the tropics, it must be capable of surviving at 29° C. Similarly, the animals of the Arctic Ocean must survive at 0° and, indeed, *Cyanea arctica* continues to pulsate even when half its bell is frozen into the ice, and after being embedded solidly for several hours it revives at once, apparently uninjured, when the ice has melted.

It is easy to see why such forms as the rhizostomous scyphomedusa *Cassiopea* and the sea-urchin *Diadema setosum* are confined to the tropics, for they lose all power of movement at from 10° to 12° C. Also the floating barnacles, *Lepas fascicularis*, can not survive in arctic waters, for they are unable to move if cooled to 4.6° C., and, conversely, *Cyanea arctica* can not enter the tropics, for it dies at 27° C.

On the other hand, even the tropical *Limulus polyphemus*, from the Marquesas, Florida, survives being frozen into the ice, and near the northern limit of its range, off the northern coast of Massachusetts, it continues to move until heated to at least 40°. Judging from its marked adaptability to extremes of temperatures, one would expect this animal to be of world-wide distribution, yet it ranges only from Maine to Yucatan and is unknown from European coasts.

When a boreal or arctic animal such as *Aurellia* becomes acclimated to the tropics, its upper death-temperature is raised, and conversely it becomes unable to withstand a degree of cold in which its northern relatives may thrive. Its optimum temperature is, however, raised even more conspicuously than its death-points and thus its factor of safety against abnormally high temperatures is reduced.

These facts are illustrated in fig. 8, page 18, wherein the ordinates of the curve *ABC* represent the average rates of pulsation of *Aurellia* at Halifax, Nova Scotia, the abscissas representing the respective temperatures in degrees centigrade. The curve *DEF* shows the same factors for the *Aurellia* from Tortugas, Florida, and it is evident that at Halifax a wide range of temperature, from 2° to 19° C., produces but little change in the rate of the medusa, whereas at Tortug a the medusa lives constantly at the temperature of its maximum activity and any further elevation of temperature causes a marked decline in its rate of pulsation.

The case of the brittle-stars *Ophioderma brevispina* and *O. appressa* is unlike that of *Aurellia*, for at Montego Bay, Jamaica, they both have the same temperature-range, their movements ceasing at 7° to 8° C. in cooled and 37.3° to 38° C. in heated sea-water; yet the former species ranges from Woods Hole, Massachusetts, to the West Indies, and the latter is restricted to the tropics.

An even more striking case is that of the tropical reef-flat coral *Siderastræa radians*, which, although it normally lives in water of 29° to 31° C., can survive without apparent injury after being placed for 8 hours in water at 6.7° C., and one specimen survived with some maceration of tissue a temperature of 1.9° C. It is difficult to see why this coral does not invade the waters of the temperate regions, but it is unknown north of Bermuda.

The development of the ability to withstand higher or lower temperatures is associated with physical changes in the protoplasm. For example, Dallinger (1887), Davenport and Castle (1895), Greely (1901), McGill (1908), Rautmann (1909), etc., observed that organisms when heated excrete water and thus their albumens become denser and the temperature of coagulation is raised. A decided congelation of the slime is observed in *Cassiopea* which has been cooled to its non-active limit.

In the tropical scyphomedusa *Cassiopea xamachana*, however, movements cease long before heat-rigor develops, and in fact the loss of movement is due to a general *muscular* relaxation, which develops several degrees below the temperature at which the nerves cease to transmit the pulsation stimulus. If, for example, we cut a ring (fig. 1) of subumbrella tissue of *Cassiopea*, leaving a radial strip projecting from the outer side and then start a pulsation-wave in the ring, every time the wave passes the point *B* a portion of the wave is diverted and passes out to the end of the strip *BC*. If, then, we place the strip *BC* and the ring in separate dishes of sea-water, and warm the ring while the strip remains at a normal temperature, the amplitude of pulsation is reduced to insensibility in the ring at about 38.5° C., and all the muscles are relaxed while the nervous impulse which produces pulsation still causes the strip *BC* to respond vigorously every time it passes the point *B* up to about 41.7° C. Thus the muscles are more profoundly affected by the heat than are the nerves.

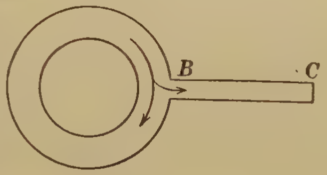


FIG. 1.

There is another reason for the belief that heat-rigor, or at least coagulation of albumens, is not the cause of death at high temperatures, for practically no marine animals can withstand 46° C., most of them dying below 40°, while the most readily coagulated emulsion of egg albumen does not congeal below 56° C.[1]

Snyder (1907, Archiv für Anat. und Physiol., p. 113), basing his conclusions upon Nicolai's observations of 1901, came to the conclusion that as many physiological reactions have the same temperature coefficient as have chemical reactions, the underlying cause of the physiological reactions must be of a chemical rather than a physical nature. In 1908 Snyder published an extensive and important paper upon this subject in which he made use of the results of all previous studies bearing upon the case; and he concludes that the temperature coëfficients of all physiological reactions in which metabolism occurs are of the magnitude of the coefficients for chemical reactions, being about 2 or 3.

---

[1] As a result of our recent experiments upon the corals of the great Barrier Reef of Queensland it appears that high temperature causes death through asphyxiation, the oxygen of the sea-water becoming insufficient to support the increased metabolism.

The equation for chemical reactions at increasing temperatures may be expressed in the form $y = ac^x$, where y is the rate of the reaction at any 10° C. temperature interval, a is the rate of reaction at the lowest temperature in the series; c is the temperature coefficient, the value of which is usually between 2 and 3; and x is the number of the 10° C. temperature intervals. Thus, if the temperature be raised 10° C., then $x = 1$; if the temperature be raised 30° C. above the lowest temperature in the series, then $x = 3$. Hence, x is the increment of increase in temperature expressed in terms of units of 10° C.

Harvey (1911) tested this subject for the rate of nerve-conduction in *Cassiopea* by experimenting upon two subumbrella rings which were deprived of rhopalia and set into pulsation by means of an entrapped circuit-wave. In being heated from 18° to about 30° C. the rate of pulsation increased in arithmetical proportion to the increment of temperature, in other words, as a rectilinear function of the increase in temperature, not as an exponential function as demanded by Snyder's hypothesis.

As Harvey was able to obtain only one wholly satisfactory series of observations, it seemed desirable to repeat his experiments. This has been done upon five separate subumbrella rings of *Cassiopea*, the results being presented in table 1, which gives the rates of pulsation of five rings of subumbrella tissue of *Cassiopea xamachana* warmed in sea-water, at Tortugas, Florida.

TABLE 1.

Temperature of the sea-water.	Number of times per minute the wave traveled around the ring.					Temperature of the sea-water.	Number of times per minute the wave traveled around the ring.				
	Ring A.[1]	Ring B.	Ring C.	Ring D.	Ring E.		Ring A.[1]	Ring B.	Ring C.	Ring D.	Ring E.
°*C.*						°*C.*					
17.5	54.5	....	....	....	....	35.07	....	....	105	....	....
20.2	60	....	....	....	....	35.1	140	....	....	....	....
21.6	70	....	....	....	....	35.22	....	....	....	180	....
24.5	82.5	....	....	....	....	35.47	....	....	....	....	164
26.6	101.5	....	....	....	....	35.95	....	....	....	180	....
27	105	....	....	....	....	36.05	....	....	105.5	....	....
27.2	....	....	....	127	....	36.3	....	136	....	....	....
27.8	....	100	....	....	....	36.75	153	....	....	....	....
28.9	111	....	....	....	....	36.85	....	....	....	183	....
29	....	....	....	....	140	27.03	....	....	103	....	....
29.3	....	....	87	....	....	37.05	....	....	....	....	154.5
30.9	121.5	....	....	....	....	37.4	....	134	....	180[2]	....
31.25	....	....	....	163.5	....	37.65	....	....	124.5		....
32.25	....	....	101.5	....	....	37.7	....	164	....	....	....
32.82	....	....	....	158	....	37.9	167	....	....	....	150
32.95	131	....	....	....	....	38.2	....	155[3]	....	....	....
33.15	....	....	102.5	....	....	38.3	166		....	....	....
34.12	....	....	....	174	....	38.5	....	....	122	....	143[5]
34.15	....	....	105	....	....	38.6	....	....	....	....	
34.3	....	126	....	....	....	39	173[4]	....	....	....	....

[1] Ring A was first cooled from 27.3° to 17.5°, its rate becoming lower as follows: 27.3° C., 70 per minute; 26°, 68 per minute; 24.5°, 72 per minute; 22°, 67.5 per minute; 20.3°, 64 per minute; 17.5°, 54.5 per minute. (See Record 1A.)

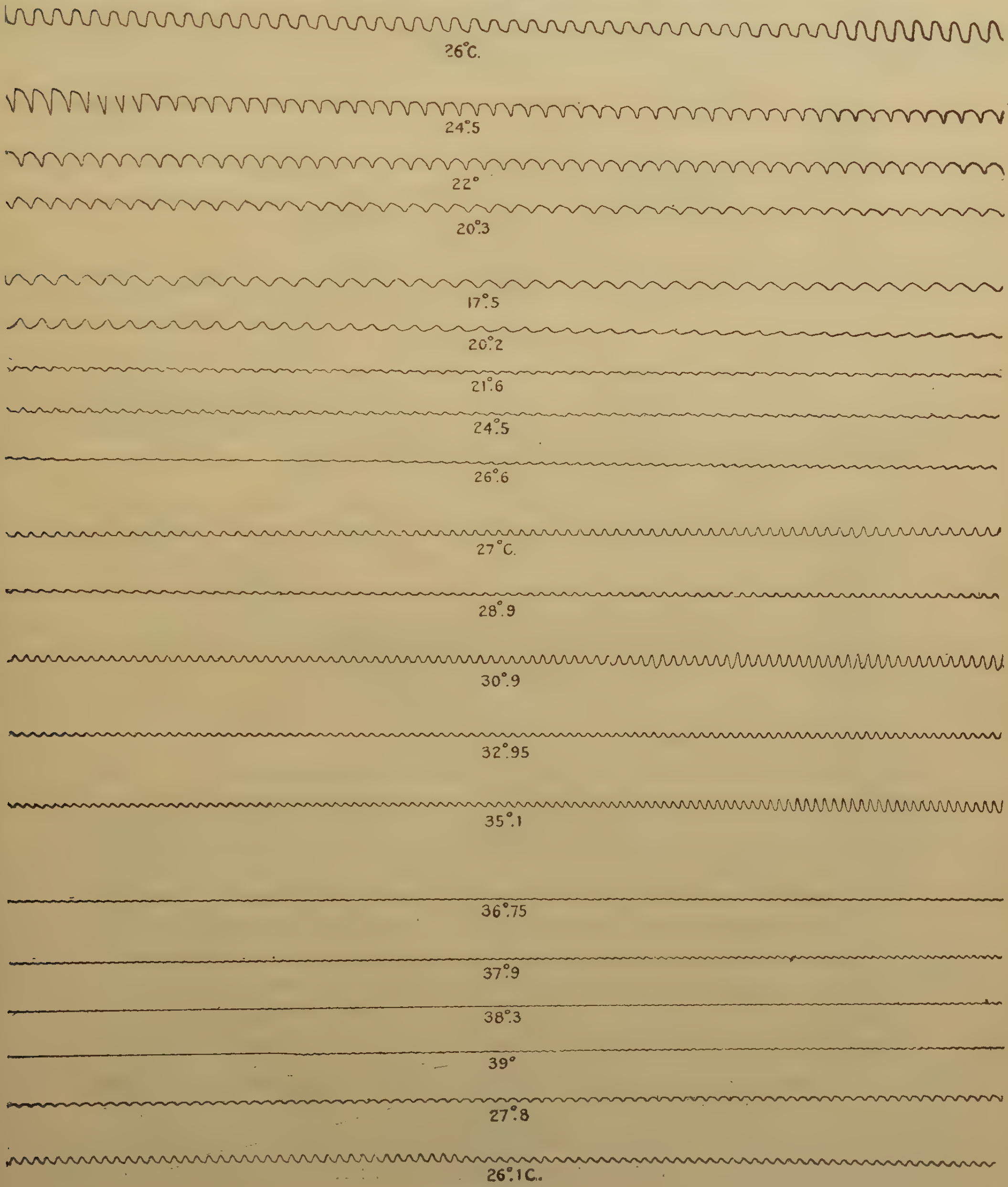
[2] This ring was finally heated to 38.75° and then upon being cooled to 28.5° it gave 89 pulsations per minute, being somewhat weaker than the normal.

[3] Upon being cooled to 30.5° this ring gave 126 turns per minute. Pulsating normally.

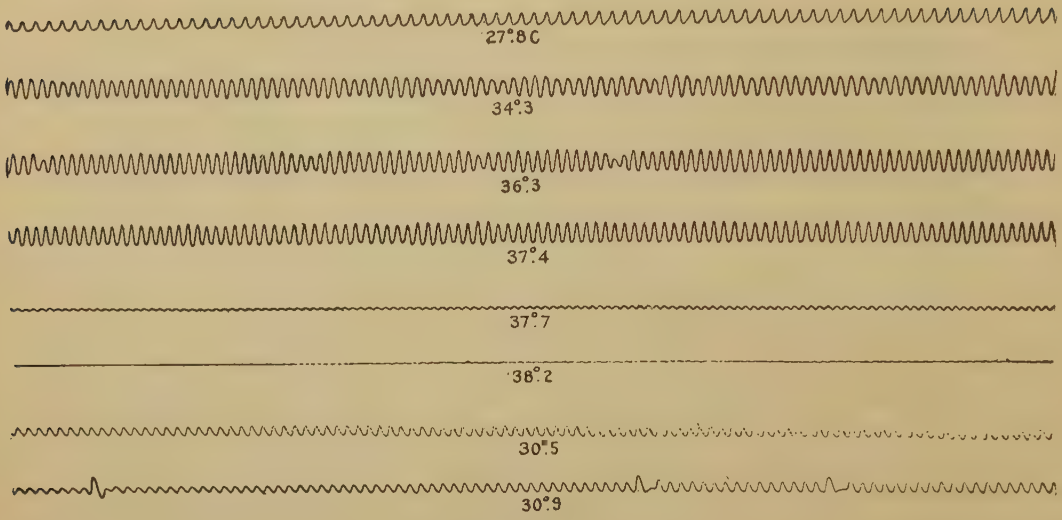
[4] Upon being cooled to 26.1° this ring gave 116 per minute. Fully recovered.

[5] Upon cooling to 29.05° the rate became 120.5°.

The most successful experiment was upon Ring A, the wave within which traveled around the ring 70 times per minute at 27.3° C., and gradually declined to 54.5 at 17.5° C., from which temperature it was slowly heated to 39° C., its rate then becoming 173. Then, upon cooling the ring to 26.1° C., it pulsated in a normal manner at the rate of 116 per minute, showing no ill effects from the experiment (see Record 1A). The obser-



Record No. 1 A.



RECORD No. 1 B.

vations are plotted in figure 2, and it appears that the increase in rate is in the form of a straight line from 17.5° C. to 35° C. In other words, it is arithmetically proportional to the increase in temperature.

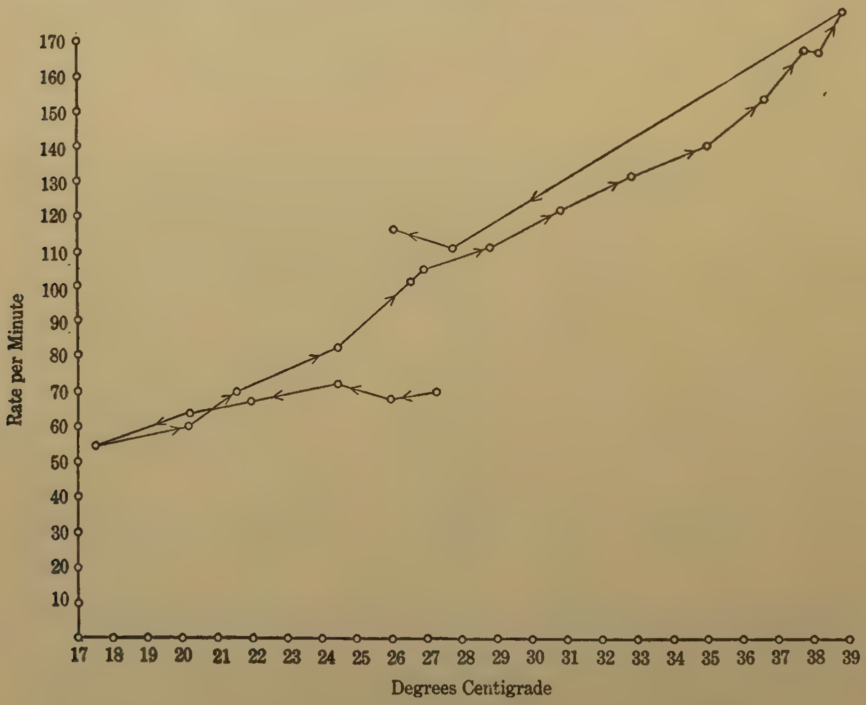


FIG. 2, illustrating Record No. 1 A.

This result is confirmed by taking the average of all five rings; for here also the increase in rate is in the form of a straight line practically coinciding with that displayed by Ring A. This average curve is shown in figure 3. The equation for the increase in rate with elevating temperature between 17.5° to 35° C., in these rings, is $y = 4.85x + 54.5$ and for Harvey's ring it is $y = 6.66x + 50$; where y is the rate at any given temperature above 17.5°, but below 30° in Harvey's and 35° on my curves. x is the temperature-increment. Thus at 25.5° C. $x = (25.5 - 17.5) = 8$, and at 27.5° C. $x = 10$, etc. The constants 54.5 and 50 are the rates of the rings at 17.5° C.

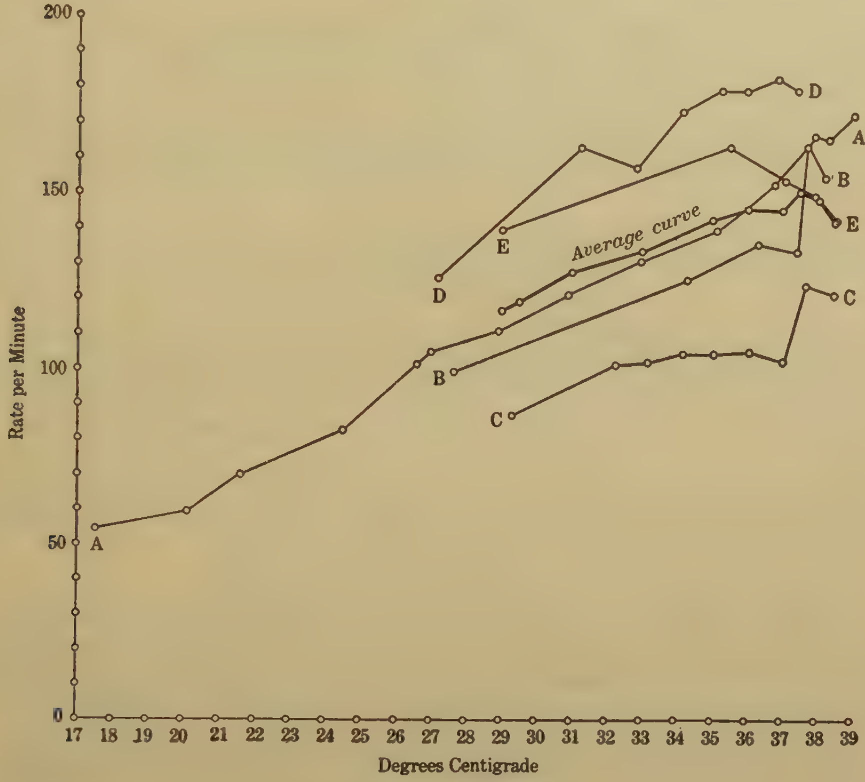
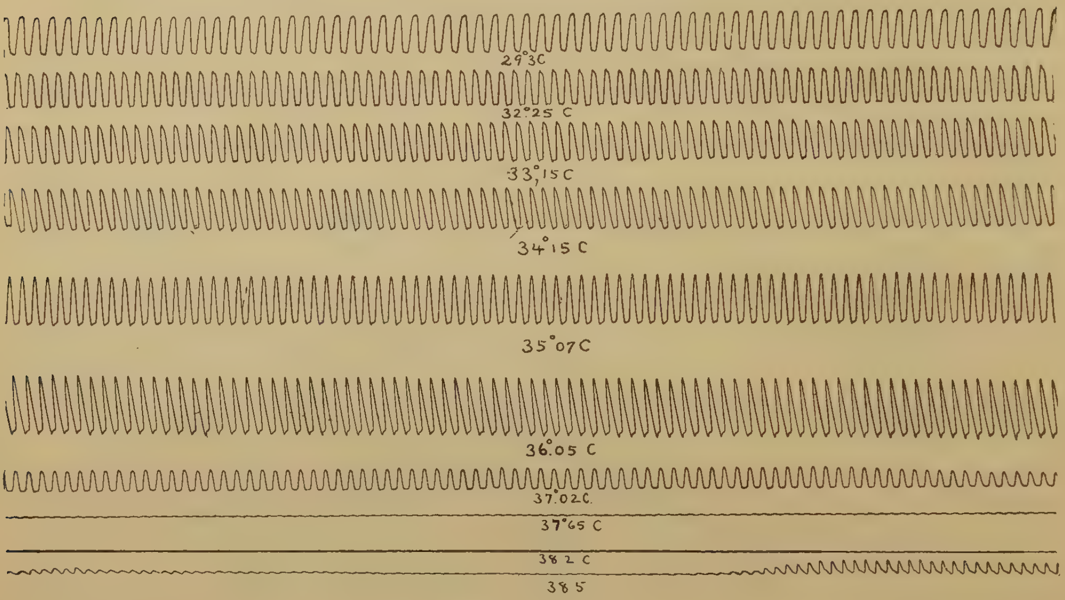


FIG. 3, illustrating Table I.

Above 33° C., Harvey observed a sudden decline in rate, but my rings did not display this decline until they had been heated to 35.5° to 39°, and Ring A did not display it even at 39° C.

As Harvey states, these curves bear a close, although possibly only a superficial, resemblance to those of enzyme actions.

Certainly our observations are not in accord with the conditions necessitated were the rate of the nervous impulse a simple chemical reaction, for in this case the curve would be given by an equation of the form $y = ac^x$, where a is the rate of 17.5° C., and c is the temperature coefficient. The actual equation is, however, of the form $y = a + x(c + 1)$.



RECORD No. 1 C.

In this connection it is interesting to observe that Knowlton and Starling (1912) find that in the excised hearts of dogs and cats the increase in the rate of pulsation between 24° and 40° C. is in a straight line, the increase in rate being arithmetically proportional to the increment of temperature. They also comment upon the inadequacy of Snyder's hypothesis to meet the conditions of their determinations, and this is the more remarkable because many physiological reactions do unquestionably follow Snyder's law.

A relation which may possibly be of some significance in this connection is that the kinetic energy of the sea-water increases, as does the absolute temperature, the increment of increase being arithmetically proportional to the temperature increment, thus increasing in a simple linear ratio, as does the rate of pulsation. The kinetic energy increases, however, at a much lower rate than does the rate of nerve-conduction. Thus, between 17.5° and 35° C. the rate of nerve-conduction has increased in an arithmetical ratio from 54.5 to about 140, or to 2.568 times its initial rate.

The kinetic energy has in the same temperature interval increased from K to

$$\frac{273 + 35}{273 + 17.5} K,$$

or from 1 to 1.06. Thus the rate of nerve-conduction has increased from

54.5 to 140, or 85.5 per minute, the kinetic energy in the same interval having increased by 0.06.

The equation for the rate of nerve-conduction at any temperature between 17.5° C. and 35° C. may therefore be expressed by

$$y = \frac{85.5}{0.06}\left[\left(\frac{273 + 17.5 + x}{273 + 17.5}\right) - 1\right] + 54.5$$

or

$$y = 1425\left[\left(\frac{290.5 + x}{290.5}\right) - 1\right] + 54.5$$

or, adopting general terms,

$$y = 1425\left[\left(\frac{T_1 + x}{T_1}\right) - 1\right] + 54.5.$$

Wherein T_1 is the absolute temperature of the lowest point of the series and x is the temperature increment corresponding to the rate y, as has been explained on page 11.

If we let 1 represent the rate of nerve-conduction and also the kinetic energy of the solution at the lowest temperature of the series, then:

$$\frac{\text{The increase in rate}}{\text{The increase in energy}} = \frac{2.568 - 1}{1.06 - 1} = 26.13.$$

Thus the increment of increase in rate of nerve-conduction, for any elevation in temperature between 17.5° and 35° C., is 26.13 times the increment of increase in kinetic energy of the solution.

Hitherto we have been considering the rate at which the nerve-net conducts the stimulus which produces muscular contraction. In nature the stimuli which produce the nerve-impulse arise in the motor centers or marginal sense-organs of the scyphomedusa and, as explained by Mayer, 1908, p. 119, the pulsation-waves annul themselves after each systole; thus the rate of pulsation in the normal medusa is much lower than the rate of nerve-conduction, being, indeed, determined solely by the rate at which the motor centers can generate successive stimuli. In the paralyzed ring, on the other hand, the rate of pulsation is simply that of nerve-conduction, for no new stimuli are generated.

The effect of temperature upon the perfect medusa is therefore its effect upon the stimulus-generating ability of the motor centers—in other words upon the rate of the metabolic process which produces stimulation. We have, then, in *Cassiopea* a unique opportunity for differentiating between the rates of production and that of the conduction of nervous impulses.

Cassiopea xamachana with motor centers intact pulsates at its maximum rate at about 33° C., ceasing to pulsate if cooled to 16.6° or if heated to 38.5°.

Table 2 gives the mean rates of pulsation derived from the study of 7 separate medusæ (with marginal sense-organs intact) at various temperatures, the rates stated in the table being the arithmetical mean of the observed rates. Thus if 3 medusæ each pulsated at the rate of 3 per minute, and if 4 others each pulsated at the rate of 10 per minute, the average rate of pulsation would be 7.

TABLE 2.

Temperature of sea-water.	No. of pulsations per minute.	Temperature of sea-water.	No. of pulsations per minute.	Temperature of sea-water.	No. of pulsations per minute.
° *C.*		° *C.*		° *C.*	
16.6	0	24	18.8	32	31
17	2.5	25	21.96	33	33.9
18	5.5	26	23.4	34	33.25
19	9.6	27	25.3	35	30.25
20	12.7	28	28.2	36	32.4
21	15.8	29	30.8	37	23.06
22	17.4	30	31	38	13.8
23	18.2	31	30.9	38.5	5.25

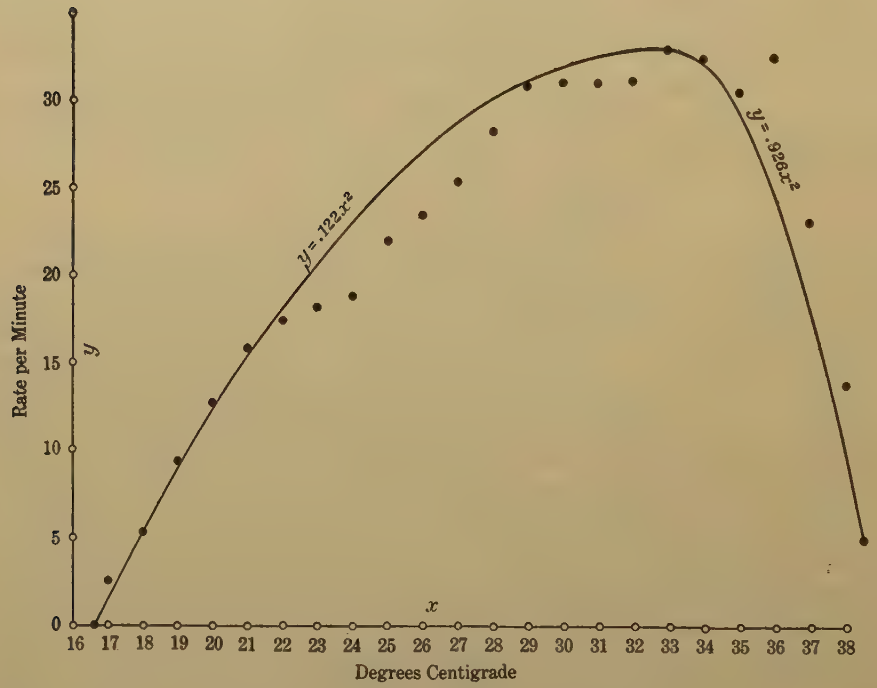
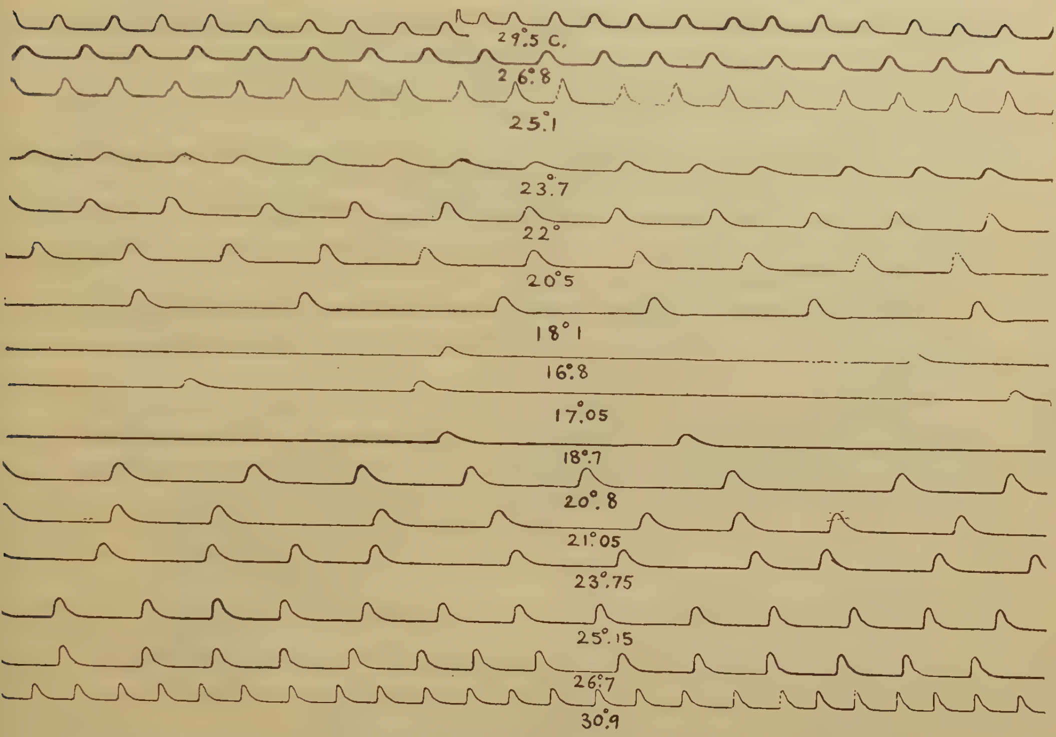


FIG. 4, illustrating Table 2.

The observations recorded in table 2 are plotted in figure 4, wherein it appears that the rate at which the motor-centers engender stimuli declines if the medusa be cooled or heated from 33°. In both heating or cooling the loss of rate is slight at first, but constantly augments as we continue to cool or heat the animal. In fact, the loss of rate is about proportional to



RECORD NO. 2.—A *Cassiopea xamachana* cooled from 29.5° to 16.8° C. and then warmed to 30.9° C,

the square of the departure of the temperature from its optimum of 33°. Thus a change in temperature above or below the optimum appears to reduce the rate of pulsation in proportion to the square of the change. For example, if cooling 1° C. produces a loss of rate of 1 per minute, a cooling of 2° from the optimum will produce a loss of 4, and for 3° the loss will be about 9, etc.

Thus if x be the departure of temperature from the optimum of 33° (for example at 30° or at 36°, $x = 3$), and if y be the loss in rate (for example, if the rate has declined from 34 to 30, $y = 4$), then in cooling $y = 0.122x^2$, and in heating $y = 0.926x^2$.

It seems that the loss of rate in heating is nearly 8 times as rapid as in cooling. Moreover, when the medusa has lost all movement through being heated, its muscles are relaxed and recovery rarely occurs, even if the medusa be then cooled to a normal temperature. If, however, the medusa be cooled to a standstill, the muscles still exhibit a decided tonus, some shrinkage occurs, and the slime congeals into a gelatinous mass.

The falling-off in rate that is observed when the medusa is either cooled or heated from its optimum temperature appears not to be a simple chemical reaction, for it is more rapid than can be represented by the equation

$y = ac^x$, but in all these experiments the equation $y = bx^2$ fits the observed facts fairly well, but this formula is merely tentative and should not be taken as being more than an empirical attempt to express the law. If this be the law, however, it would mean that the decline in rate varies as the square of the degree of heating or cooling above or below the optimum, and it leads one to suspect either that an inhibiting enzyme is formed in a constantly accelerated ratio or a stimulating one is destroyed in the same manner. This is, however, a mere suggestion, for the observations are too few to establish it as a law.

Experiments upon *Cassiopea frondosa* and *Aurellia aurita* at Tortugas appear to lend some support to the results attained from a study of *C. xamachana.*

Thus table 3 shows the average rates of four medusæ of *Cassiopea frondosa* at Tortugas, Florida, cooled from 28.5° to 12.5° C., and figure 5 gives a graphic representation of the results. It is evident that the rate declines somewhat more slowly than if it followed the equation $y = 0.0507x^2$.

TABLE 3.

Temperature.	Pulsations per minute.
° *C.*	
28.5	13
25	11
20	9
15	3
12.5	0

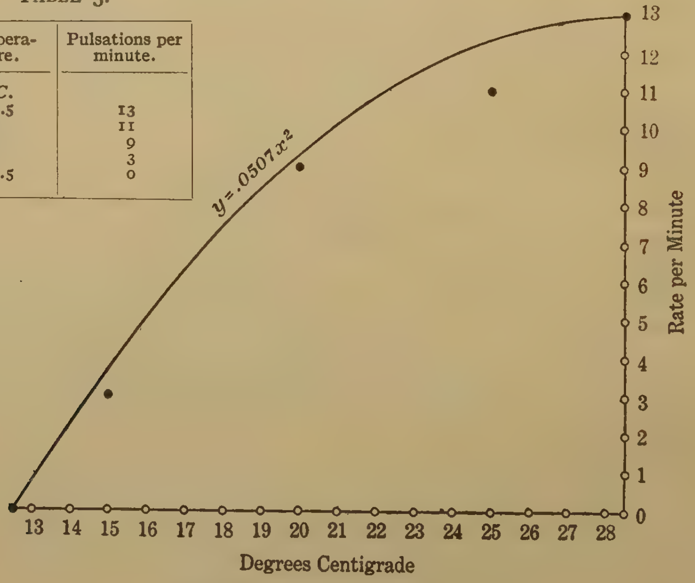


FIG. 5, illustrating Table 3.

Upon being heated, *Cassiopea frondosa* begins to lose in rate at 32.45° and all movement ceases at 38.3° C. Unfortunately, I did not make enough observations to determine the curve of the decline, but if it be a parabola its equation must be $y = 1.05x^2$, and thus the decline in rate upon heating above the optimum is about 20 times as rapid as the decline on cooling below the optimum.

Table 4 and figure 6 show the decline in rate of an *Aurellia aurita* from Tortugas, Florida, cooled from 28.8° to 11.8° C.

TABLE 4.

Temperature of sea-water.	Pulsations per minute.	Temperature of sea-water.	Pulsations per minute.
° *C.*		° *C.*	
28.8	25	14.6	12
20.5	18	13.2	8.5
18.1	17	13	1
16.4	14	11.8	0

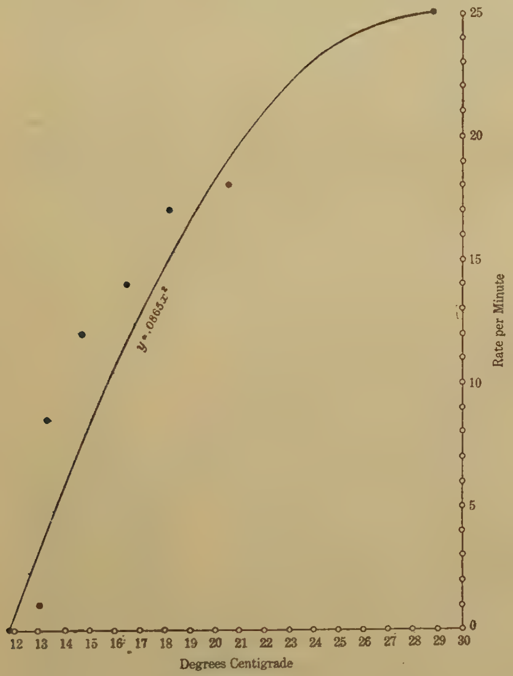


FIG. 6, illustrating Table 4.

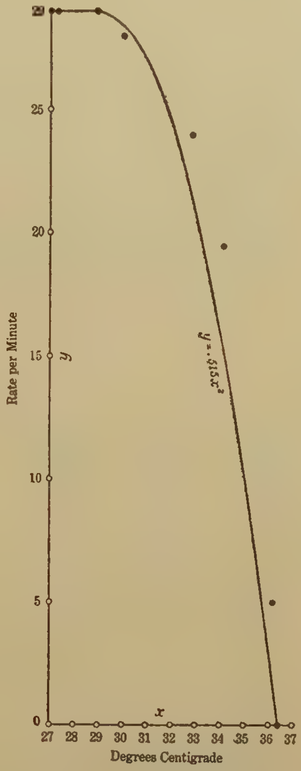


FIG. 7, illustrating Table 5.

TABLE 5.—*An Aurellia aurita at Tortugas, Florida, warmed from 27.3° to 36.4° C.*

Temperature of sea-water.	Pulsations per minute.	Temperature of sea-water.	Pulsations per minute.
° *C.*		° *C.*	
27.3	29	34.1	19
28.9	29	36.15	5
30	28	36.4	0
32.8	24		

The parabola $y = 0.0865x^2$ fits the observed facts fairly well.

Similarly, table 5 and figure 7 show the decline in rate displayed by a specimen of *Aurellia aurita*, from Tortugas, which was heated from 27.3° to 36.4° C., and the actual conditions are fairly well represented by the curve $y = 0.515x^2$, showing that in heating the rate declines about 6 times as rapidly as it does on cooling; the case being in general similar to that of *Cassiopea xamachana*, where the rate of decline upon heating is about 8 times as rapid as in cooling.

Hitherto we have considered only tropical forms or animals living in the tropics, and in these we see that a definite temperature of maximum activity is well shown in their temperature reactions. Thus for *C. xamachana* the optimum is about 33° C., for *C. frondosa* 28.5° to 32.5°, for *Aurellia aurita* 29°, for the movements of the branchial arms of *Lepas fascicularis* it is about 32° C., and for the reef corals it ranges between 30° and 35.7° C. Thus the optimum temperature is very close to the usual temperature of the sea-water itself, this being about 28° to 31° in summer. Even a slight degree of cooling or heating beyond the optimum causes a decided falling-off in rate.

A very different picture is presented by the arctic scyphomedusa *Cyanea arctica*, from Halifax, Nova Scotia, in September, for here the optimum temperature ranges from about 2° to 21° C., the animal's rate of pulsation increasing only slightly as the temperature rises, and with an ill-defined maximum at about 19° C. Upon cooling below 2° C., the medusa pulsates until the ice imprisons it, although its rate declines rapidly. Similarly, if we heat it above 21° C. the rate declines at about the same rate as it does upon cooling from 2° C. All movements cease at about 27° C.

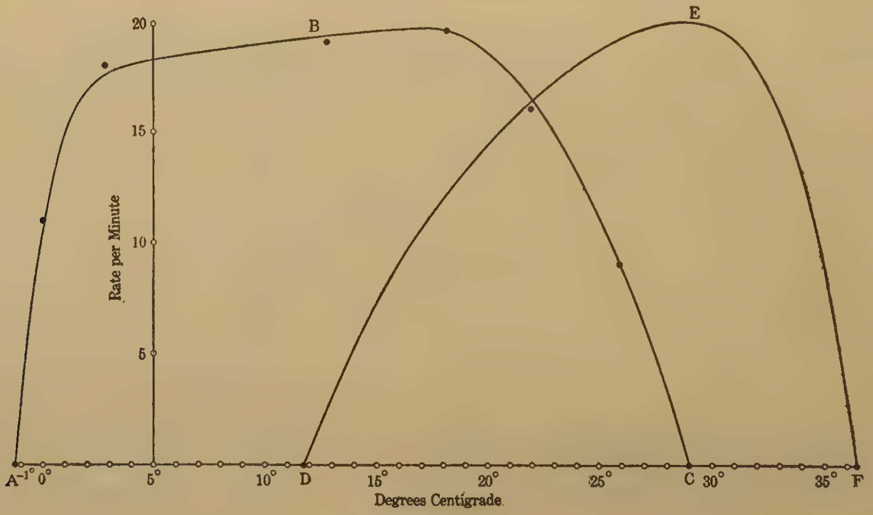


FIG. 8.

The same general conditions are also shown by *Aurellia aurita* from the cold waters of Nova Scotia, in marked contrast with its behavior at Tortugas. For example, at Halifax, Nova Scotia, in September, when the harbor water is about 14° C., the optimum temperature for *Aurellia* is anywhere from 2° to 18° C. and it ceases to pulsate at −1.4° and at 29.4° C.

At Tortugas, on the other hand, a very slight departure in temperature either above or below 29° C. causes a decided falling-off in rate.

The contrast between the behavior of *Aurellia* at Tortugas and at Halifax is shown in figure 8 wherein the ordinates represent average rates of pulsation and the abscissæ temperatures, the curve *ABC* applying to the medusæ from Halifax, while *FDE* shows the general reactions of the same species from the warm waters of Tortugas, Florida.

The wide range in temperature in which these northern forms are about normal or only slightly increasing in activity accords with the fact that tropical marine animals are, physiologically speaking, but poorly adjusted to their temperature environment in comparison with creatures of colder waters, for the tropical forms live near their upper death-points and slight elevation in temperature affects them adversely. Nor are they capable of withstanding the same proportionate degree of cold as do northern forms, as may be seen after every severe "norther" in winter, when the Florida beaches are strewn with reef fishes which have succumbed to the cold, although the temperature of the water over the reefs in these storms rarely declines below 17° C.

TABLE 6.

Name of coral.	Temperature at which death occurs.	Lowest temperature survived without apparent injury.	Temperature at which tentacles were most fully expanded.	Temperature at which tentacles retracted and movements ceased.	Highest temperature survived without apparent injury.	Temperature at which death occurs.
	°C.			°C.	°C.	°C.
Siderastræa radians[1]	4.5	1.9 to 6.7	32.5 to 35.7	35.7	38.2	38.5
Siderastræa siderea[2]	5.1	11.3	....	....	37.8	38.3
Mæandra æreolata[3]	....	10.2	30 to 34.2	31.8 to 35.2	35.9	36.7
Mæandra clivosa[4]	....	15.3	31.3	34.4	37.7	38.2
Manicinia gyrosa	....	....	....	....	36.1	37.8
Porites furcata[5]	....	3 hrs. at	32.65	36.4	38.2	37.2 to 38.3
Porites clavaria[6]	....	10.2	32.8	34.5 to 34.9	36.7	37.7
Porites astræoides[7]	15.3	....	33.7	34.3	36.7	37.7
Favia fragum[8]	....	....	....	34.7	36.1	37.2
Orbicella annularis[9]	14.1	16	....	....	36.2	36.8
Orbicella cavernosa	above 8.4	....	....	....	....	below 37.3
Agaricia sp.	....	....	....	....	....	35.95
Mussa (Isophyllia) dipsacea	....	....	....	....	....	36.7
Mussa (Isophyllia) rigida	....	....	....	....	....	35.95
Oculina diffusa	....	15.6	....	....	36.1	36.4
Madrepora (Acropora) muricata[10]	....	14.1	33.75	34.7	36.8	35.8 to 37
Madrepora (Acropora) palmata	....	....	33.75	34.7	35.7	35.8
Eusmilia aspera (knorri)	17	....	....	....	36.1	36.4

[1] Survives without apparent injury after being for 9 hours at 11.2° to 12.1° C.
[2] Survives 13° to 14.4° C. for 9 hours; half killed by 11.2° to 12.1° for 9 hours.
[3] Apparently uninjured by 13.2–15.1° for 10.5 hours.
[4] Killed by 13° to 14.4° for 9 hours.
[5] Nearly killed by 13.2° to 15.1° for 10.5 hours.
[6] Not injured by 13.85° to 16.8° for 9 hours.
[7] Killed by 14° to 15° for 9 hours.
[8] Injured but survived 13.85° to 16.8° for 9 hours.
[9] Killed by 14° to 15.8° for 9 hours.
[10] Killed by 13.3° to 15° C. for 9 hours.

The reef corals are interesting, for, as is well known, they are restricted to tropical seas. Accordingly, the vital limits in respect to temperature of the 18 most abundant reef species of the Florida-Bahama region were studied.[1] The results are presented in table 6, the method pursued being the same as in all other experiments recorded in this paper: the corals were placed in large glass aquaria and the temperature was raised slowly at the rate of about 2° C. per hour, with frequent stirring of the water. Time is an important factor in these experiments, for animals can withstand a higher degree of heat if the temperature be raised quickly than if it be raised slowly. These experiments were made during May–July 1912, at Golding Cay, Andros Island, Bahamas, and at Tortugas, Florida, when the normal temperature of the sea-water ranged from 27° to 29.5° C. as shown in table 6.

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[1] Experiments upon corals of the Great Barrier Reef of Australia, near Torres Straits, show that their temperature reactions are on the whole similar to those of the corresponding Atlantic genera. Thus natural selection has not aided the Florida corals in withstanding cold, or improved the resisting powers of the Pacific forms in respect to heat.

It appears that the reef corals at Tortugas, Florida, live in water which is commonly within 10° C. of their upper death-temperature, and if the ocean were heated to 38° C. (100.4° F.) only one species, *Siderastræa radians*, could survive. This form lives on the shallow flats, often in places where the circulation is imperfect and where wide temperature ranges occur. Accordingly, it is also the species most resistant to cold, withstanding 6° to 7° C. without apparent injury, but usually being killed at about 4.5° C. (40° F.), although one individual survived without apparent injury after being at a temperature of 1.9° C. (35.4° F.) for 11 hours.

Next to *Siderastræa radians* the most resistant coral is *S. siderea*, although it lives in relatively deep water on the outer reefs, where the circulation is of the best and the temperature range is therefore slight. It is associated in its habitat with *Orbicella annularis*, one of the most sensitive of the reef corals, which is killed at 14.1° and 36.8° C.

In general, however, the corals of the shallow-reef flats, such as *Siderastræa radians*, *Porites furcata*, and *Mæandra areolata*, are the most resistant both to heat and cold, while those of deep water, such as *Madrepora palmata*, *Eusmilia knorri*, and *Oculina diffusa*, are the least resistant.

Forms such as *Porites clavaria*, *P. astræoides*, *Mæandra muricata*, *Orbicella annularis*, *O. cavernosa*, and *Favia fragum*, which usually live in fairly shallow but freely circulating water, all show moderate powers of resistance.

The air-temperature at Tortugas commonly ranges from a maximum of 98° F. in summer to about 60° F. in winter,[1] and the coldest "northers" in winter appear to reduce the temperature of the water over the reefs to about 17.2° C. (63° F.). In view of this fact, the more abundant reef species were maintained at about 13.9° C. (57° F.) for periods of at least 9 hours.

As a result, we are led to conclude that were the water cooled by an exceptionally prolonged norther to 13.9° C. for 9 hours, *Siderastræa radians*, *S. siderea*, and *Mæandra areolata* would survive without apparent injury, while *Porites furcata*, *P. clavaria*, *Mæandra clivosa*, and *Favia fragum* would also survive, but with more or less injury, the first-named being the most resistant. On the other hand, this temperature would be fatal to *Orbicella annularis*, *Porites astræoides*, and *Madrepora muricata* (*cervicornis*).

The observations cited above are upon the death-temperatures, but the temperatures at which the feeding reactions and normal metabolic processes cease are much more significant, for naturally an animal can not long survive in water in which it can neither move nor function. Unfortunately, however, no observations were made upon this interesting point; but, crude as they are, the experiments show why it is that most of these reef corals can not enter the waters of the temperate regions and demonstrate that, in common with other marine animals, they live at temperatures within about 5° of their temperature of maximum activity and within 10° of their death temperatures. Thus the factor of safety in respect to elevation of temperature is far less in tropical than in temperate marine animals, and they are, relatively speaking, poorly adjusted in a physiological sense to their temperature environment; slight differences in temperature producing a more serious effect than is observed to result from similar temperature changes with the marine animals of the temperate regions. Moreover, paradoxical as it may seem, tropical marine animals can withstand cooling better than they can survive heating above their normal life-temperature. High temperature appears to cause death by asphyxiation, the oxygen of the seawater becoming insufficient to support the augmented metabolic activity of the animal.

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[1] In the year from June 1, 1912, to May 31, 1913, the air temperature at Tortugas ranged from 95° to 65.5° F. as determined by a self-recording thermometer.

TABLE 7.—*Reactions to Temperature.*

Name of animal.	Cold.		Temperature of greatest activity.	Heat.		Normal temperature of sea-water in which the animals were living.
	Death occurs.	Movements cease.		Movements cease.	Death occurs.	° C.
Aurellia aurita:						
From Tortugas, Florida, June–July 1911.	Killed if ice crystals form within gelatinous substance of medusa......	7.75° to 11.8° C......	About 29° C........	36.4° to 38.4° C.....	38.5° C.	29
From Halifax, Nova Scotia, September 1911.	Survives being frozen solidly into the ice.	− 1.4° C. with ice crystals forming in water.............	18° to 23° C........	29° to 29.7° C.......	29° to 30° C.: usually survive if heated not above 29° C.	14
Cyanea arctica from Halifax, Nova Scotia, September 1911.	Survives freezing solidly into ice without apparent injury, being more resistant than *Aurellia.*	Below − 1.4° C. while ice crystals are forming in water.	About 19° C........	26.8° to 28° C.......	26.8° to 28° C........	14
Pennaria tiarella:						
From Tortugas, Florida, July 1911.	Killed by freezing to −2.3° C.	....	....	....	....	29
From Montego Bay, Jamaica, March.	−0.6° C.	....	....	34.7° C...........	....	22 to 26
From Woods Hole, Massachusetts, Aug. 1911.	Survives freezing to −2.3°.	....	....	....	....	22
Lepas fascicularis from Tortugas, Florida, June–July 1911.	Killed between −1.4° and −2.3° C. while ice crystals form in the water. About half of specimens recover from −1.4° C.	4.6° to 11.5° C........	30.3° to 32.1° C.....	38.4° to 40.7° C.....	42.3° C.............	29
Limulus polyphemus from Marquesas Keys, Florida, July 1911.	Survives −2.3° C. with ice forming in water.	+0.8° C............	41° C.............	45.7° C............	46.25° C............	30
From Annisquam, Mass., Oct. 1, 1911.	Survives being frozen into a solid block of ice and then thawed out.	Below −1.1° C. slow movements continue while ice crystals are forming in water....	....	40° C.............	41° C..............	16
From Woods Hole, Mass., Aug. 1911.	....	....	38.1° C...........	41° C.............	41° C..............	22
Beroë cucumis, from Halifax, Nova Scotia, Sept. 1911.	....	....	18° to 28° C........	27.8° to 29.8° C.....	29.7° to 30° C.......	14
Cassiopea frondosa, from Tortugas, Florida, July 1911.	8.3° to 9.7° C..........	12.1° to 12.9° C......	About 32.5° C......	38.3° to 40° C.......	40° C..............	29

TABLE 7.—*Reactions to Temperature.—Continued.*

Name of animal.	Cold.		Temperature of greatest activity.	Heat.		Normal temperature of sea-water in which the animals were living.
	Death occurs.	Movements cease.		Movements cease.	Death occurs.	° C.
Diadema setosum, from Tortugas, Florida, July–Aug. 1911.	+2.6° C. The spines recover temporarily when animal is warmed from this temperature, but tube-feet do not recover, so the animal dies.	Tube-feet cease to move at 10° to 10.8° C.; spines continue to move down to 5° C.	Spines are most active at 36.5° C.	Spines cease to move at 36.5° to 37.6° C.; tube-feet cease at 35.5° C.	37.4° to 37.6° C.; spines recover temporarily but tube-feet do not, so animal dies.	29
Larvæ of Atlantic palolo worm (*Eunice fucata*) 4 or 5 days old, from Tortugas, Florida, July 20 and 21, 1911.	Recovers from +1° to 6° C., but is killed at −2.3° C.	7° to 9.5° C.	About 35.5° C.	41° to 42.7° C.	Above 42.7° C.	29
Toxopneustes (Lytechinus) vareagatus, from Montego Bay, Jamaica, West Indies.	Below 0° C.	7° C.	24° to 36° C.	38° C.	Can not survive 37.7°C.	24.6 to 25.1
Ophioderma brevispina from Montego Bay Jamaica.[1]	− 0.6° C.	7.5° to 8° C.	About 30° C.	37.3° to 37.7° C.	37.7° C.	22.35 to 25.3
Ophioderma appressa.[2] Montego Bay, Jamaica.	....	....	....	37.2° to 37.7° C.	37.7° C.	22.3 to 25.3
Ophioderma brevicauda[2] from Montego Bay, Jamaica.	Survived 1.2° C.; killed at −1.7° C.	7.5° to 8° C.	....	....	....	....

[1] This species ranges from Woods Hole, Massachusetts, to West Indies.

[2] This species is confined to the tropics.

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II.

THE RELATION BETWEEN THE DEGREE OF CONCENTRATION OF ELECTROLYTES OF SEA-WATER AND THE RATE OF NERVE-CONDUCTION IN CASSIOPEA.

By ALFRED GOLDSBOROUGH MAYER,
Director of Department of Marine Biology of the Carnegie Institution of Washington.

Thirteen figures.

THE RELATION BETWEEN THE DEGREE OF CONCENTRATION OF THE ELECTROLYTES OF SEA-WATER AND THE RATE OF NERVE-CONDUCTION IN CASSIOPEA.

By Alfred Goldsborough Mayer.

CONCLUSIONS.

If sea-water be diluted with distilled water, or with a 0.9 molecular solution of dextrose, thus preserving its normal osmotic pressure but reducing the concentration of the cations of sodium, magnesium, calcium, and potassium, the rate of nerve-conduction increases as dilution proceeds, becoming most rapid in 90 per cent sea-water + 10 per cent distilled water or dextrose. In 80 per cent sea-water it is again about normal in rate, and in successively lowered concentrations it declines in a right-line ratio. The curve for rate of nerve-conduction in sea-water diluted with distilled water is practically identical with that for sea-water diluted with 0.9 molecular dextrose, thus showing that the changes in rate are due to changes in concentration of the electrolytes and not to osmotic pressure. (See fig. 6, page 40.)

If sea-water be diluted with a solution of 0.487 molecular sodium chloride dissolved in 0.075 molecular dextrose, thus preserving the normal concentration of sodium in sea-water and the normal osmotic pressure of the sea-water, the rate of nerve-conduction is most rapid in about 90 per cent sea-water + 10 per cent of this solution and then declines becoming about normal in rate in 80 per cent sea-water + 20 per cent of the (NaCl + dextrose) solution, after which it declines in a right-line ratio upon further dilution, but more slowly than if the sea-water had been diluted with distilled water or dextrose. Thus the sodium cation is an active stimulant for nerve-conduction. (See fig. 7, page 43.)

Similar experiments with the magnesium cation show that it is not a stimulant for nerve-conduction, being no more effective in this respect than is distilled water. Thus it is inert and nontoxic, and exhibits only negative properties and probably it should not be classed as an active inhibitor. Speaking crudely, its rôle in respect to sodium in sea-water is comparable to that of the nitrogen of the air in relation to oxygen. (See fig. 9, page 47.)

In very slight excess the potassium cation produces a permanently stimulating effect, as does sodium, but in denser concentration it produces momentary stimulation of the rate of nerve-conduction followed by depression. In all essential respects the effects of potassium are similar in kind, but more marked in degree, to those of its close chemical ally, sodium.

I can not determine the individual effects of the calcium cation owing to its habit of associating itself with the sodium, thus enabling the sodium to offset the effects of magnesium.

The laws stated above for the rate of nerve-conduction apply also with modifications of detail to the rate at which the motor centers (the rhopalia) generate the stimuli which produce the nerve-impulse. The rhopalia are, however, more readily affected by osmotic and by concentration changes than are the nerves, and I have not observed any increase in rate upon diluting the sea-water with distilled water or dextrose. Any change in concentration either above or below that of normal sea-water produces depression in the action of the rhopalia.

Attention may be directed to an explanation of the apparent converse relation between the activities of muscles and cilia in trochophores, ctenophores, and other forms having well-differentiated cilia which move in a coördinated manner. In all these cases the normal muscular tonus of the animal produces a state of tension over the outer skin, thus pressing upon the cilia-bearing cells and reducing or even stopping their movement. When this tonus is relieved, however, the cilia beat rapidly. Thus magnesium reduces the muscular tonus, but its depressant effect upon cilia is not so marked as upon the muscles, and the cilia-bearing cells being relieved of pressure beat with abnormally great activity in such a solution.

Sodium, on the other hand, contracts the muscles, thus increasing the pressure upon the cilia-bearing cells and stopping the movement of their cilia. Hence this converse relation between neuro-muscular and ciliary movement is a mechanical, not a chemical, matter.[1]

THE GENERAL NATURE OF THE PULSATION-WAVE.

Cassiopea xamachana is a very favorable animal for physiological experimentation, for it normally lives in semistagnant lagoons and is, therefore, relatively insensitive to changes in osmotic pressure due to concentration or dilution of the sea-water. Moreover, as there is probably a considerable amount of dissolved CO_2 in its normal environment, and as the medusa is infested with symbiotic plant-cells, it thrives remarkably well in aquaria, there being practically no deaths due to confinement under laboratory conditions.

In this series of experiments, whenever the animals were placed in a new solution they were permitted to remain in a large quantity of it for at least an hour with several changes before the effect of the new solution was determined by a kymograph record. In this manner the ultimate condition of penetration and balance between the animal and the surrounding solution was secured. The relative concentrations of the solutions were determined by titration with $AgNO_3$ and potassium chromate.

The effects described are all practically reversible, and fatal or seriously injurious degrees of dilution of ions were avoided, so that when the animals were returned to sea-water they soon regained most of their normal rate and amplitude of pulsation.

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[1] Mayer (1912), Ctenophores of the Atlantic Coast of North America, p. 25. Carn. Inst. Wash. Pub. No. 162.

The stimuli which cause pulsation normally arise in the motor centers called rhopalia, or marginal sense-organs, of which there are usually about 16 in *Cassiopea xamachana*, although they may vary in number from 9 to 23. It appears that this normal stimulation is due to the constant formation of sodium oxalate in the rhopalia, thus precipitating calcium oxalate to form the crystals of the sense-club and setting free ionic sodium, which is a most powerful stimulant.[1]

The pulsation-rate of the normal medusa is therefore the rate at which its motor centers can produce successive stages of stimulation. This is always much slower than the rate at which the nerves can conduct the pulsation-wave around the subumbrella of the medusa, and there is a long pause after each contraction, during which the animal remains unstimulated and hence inactive.

Cassiopea is, however, an excellent animal for physiological studies upon nerve-conduction, on account of the ease with which we may dispense with the motor centers and yet maintain a continuous neurogenic pulsation-wave in the subumbrella tissue. This method was devised and described by Mayer, 1906, p. 22, and it consists in cutting off the rhopalia, thus paralyzing the nervous network of the subumbrella. Then the subumbrella tissue is cut into the form of a ring-shaped strip, and a pulsation-wave is started in *one direction* in this ring, so that it must continue to travel around the ring, being in effect entrapped within the circuit of tissue through which it must continually progress in one direction, a single stimulus often maintaining itself in this manner for days at a time.

Such entrapped circuit-waves are of course not peculiar to *Cassiopea*, for they may be started and maintained in ring-shaped strips of the ventricle of the heart of the sea turtle, *Caretta caretta*, in the hearts of fishes, or in other scyphomedusæ. We may thus study the operations of the nervous network as a continuous transmitter of a single pulsation-stimulus.

As has been said, the nerves transmit this stimulus around the bell much more rapidly than the motor centers can engender it. For example, a medusa, which normally gave 45 pulsations per minute, was deprived of its rhopalia and cut into the form of a ring, and a pulsation-wave then traveled constantly around this ring at the rate of 129 turns per minute. This ring also responded without missing a pulsation to 152 Faradaic shocks per minute, and it followed 190 shocks, responding by a separate contraction to each one for about 3 minutes, after which it occasionally failed to respond, following alternate stimuli. Many experiments of this sort were made, always with the same general result, and it is evident that the nerves and muscles can react to successive stimuli with greater frequency and at shorter time-intervals than they are called upon to respond in nature.

It is evident, then, that there is a considerable "factor of safety" in the normal medusa, and that the motor centers initiate pulsation-stimuli at a rate much slower than that to which the nervous network and the underlying muscles can respond, and thus the danger of muscular fatigue is avoided.

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[1] Mayer, 1908, p. 130.

As has been pointed out by Romanes (1885), Bethe (1903), and Harvey (1911), there must be some mechanism of adjustment by virtue of which the wave travels at a faster rate around the rim than at points nearer the center of the subumbrella, the rate being directly proportional to the length of the radius of the zone of pulsation.

In order to determine the average rate of transmission of the pulsation-impulse by the nervous network, eight separate rings were cut, one from each of eight *Cassiopea xamachana.* These rings were each 90 mm. in outside diameter, 67 mm. in inside diameter, and 11.5 mm. wide. Thus the mean diameter of each ring was 78.5 mm. and its mean circumference 247 mm.

TABLE I.

Ring No. on record No. 1.	Diameter of medusa from which the ring was cut.	Times per minute wave went around ring.	Distance wave traveled per second.	Temperature.
	mm.		*mm.*	°
1	98	117	481	30.45 *C.*
2	110	116	478	28
7	109	113	464	29.7
*6	111	106	436	29.8
3	110	104.5	429.5	29.8
5	97	103	422	29.5
8	108	103	422	30.3
4	115	93.5	384	28.5
Av. condition	107	107	440	28.9

* This ring exhibits *pulsus alternans.* For an explanation of this phenomenon, see G. A. Mines, 1912, Proc. Cambridge Philosophical Society, vol. 16, p. 617.

The results obtained from the kymograph record, figure 1, are stated in table 1, which shows the rate of nerve transmission, in millimeters per second, determined in natural sea-water, ranging in temperature from 28° to 30.45° C.

These 8 medusæ ranged from 97 to 115 mm. in diameter, and their pulsation-waves traveled at rates varying from 384 to 481 mm. per second, the average being 440 at an average temperature of 28.9° C. In these experiments, the rate of transmission bears no relation to the size of the medusa.

All experiments show that, as in other invertebrates, strong stimuli travel faster than weak ones. Thus should a ring stop, we can not start it again and continue the same series of experiments with it, for the new stimulus is almost certain to differ in strength from the former one; or, if all care be taken to make the successive electrical stimuli as nearly as possible identical in strength, they will be received differently by the tissue and the resulting pulsation-waves will differ in rate. Thus a ring must remain in pulsation throughout the entire series of experiments that one performs upon it or no conclusions can be drawn respecting the relative rates of its pulsation-stimulus in the various solutions.

On the other hand, temperature, purity of water, etc., being unchanged, the rate remains nearly constant for any single stimulus. Thus Harvey (1911, p. 131) maintained such a ring in pulsation for 11 days at an average velocity of 774 mm. per second. I once had a ring which remained in pulsation for 6 days, the wave traveling from 200 to 206 times per minute around the ring. The rings live for two or more weeks and exhibit abortive efforts at regeneration, but being without means of obtaining food they gradually decline in size and die.

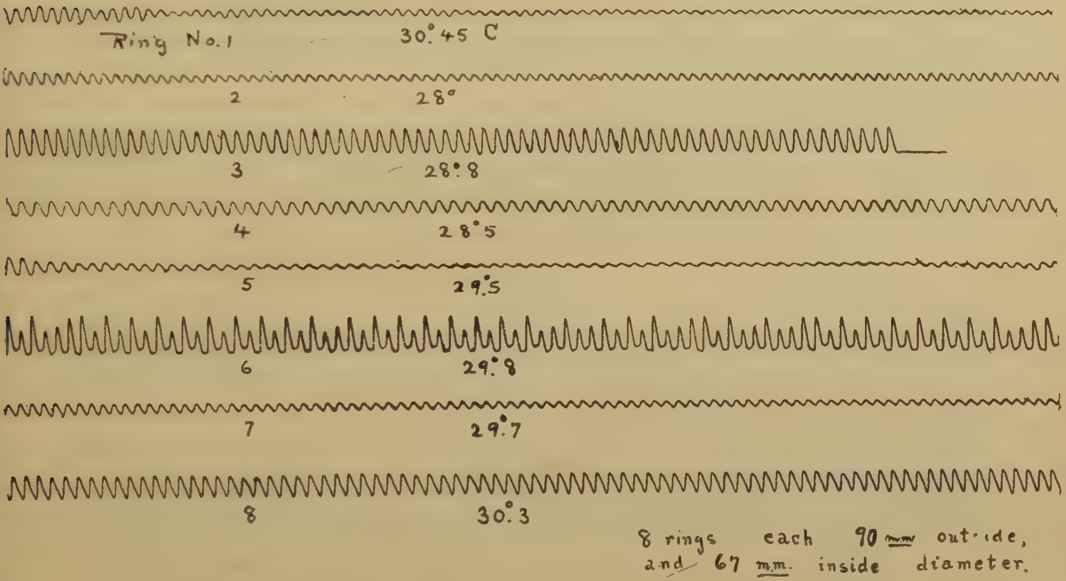

FIG. 1.

These pulsating rings are usually almost machine-like in their uniformity of rate under unchanged environmental conditions; but while the rate of nerve-conduction remains quite constant, the muscles become fatigued and thus the amplitude of pulsation steadily declines, becoming much reduced at the end of a few days. Thus the muscles fatigue more readily than do the nerves.

Kymographic records enable us to study not only the rate, but the form of the pulsation-wave, and this is important; for in order that comparisons may be made it is necessary that the character of the wave should not change throughout the series of tests to which the ring may be subjected in the course of any one set of experiments.

For example, the series of lines on the record (fig. 2), which were obtained from a single medusa, can not safely be compared one with another and the record is therefore worthless, on account of the tendency of the pulsation-stimulus to break up into two waves, the one following the other at the same or at different rates as in lines 1 and 3 from the top of the figure; or, the wave may break up into a complex series of strong and weak wavelets due to spontaneous excitement, as in line 5.

When a simple contraction-wave breaks up into two or more wavelets, the one following the other, the rate declines, for strong stimuli travel faster than weak ones; and if an originally unitary wave divides into two, the stimulus represented by each component wavelet is naturally less than that of the wave as a whole. Thus a ring which gave 168 pulsations per minute with a unitary wave gave only 132 per minute when this wave broke into two component wavelets, the one following the other around the ring. Moreover, if two waves follow one behind the other, the tissue does not enjoy so long a resting-period as if one simple wave were in the circuit, and a certain time must be permitted to pass before the muscles which have responded by a contraction can again fully respond to another stimulus. In some rings the tissue may give a strong response only to every alternate or even every third return of the wave, showing that the muscles which have very recently contracted can not again give a maximal response until a certain resting-time has elapsed. This was first observed in the muscles of Scyphomedusæ by Romanes, 1885, and has been studied in detail by Bethe (1903 and 1908). (See line 6 of fig. 1.)

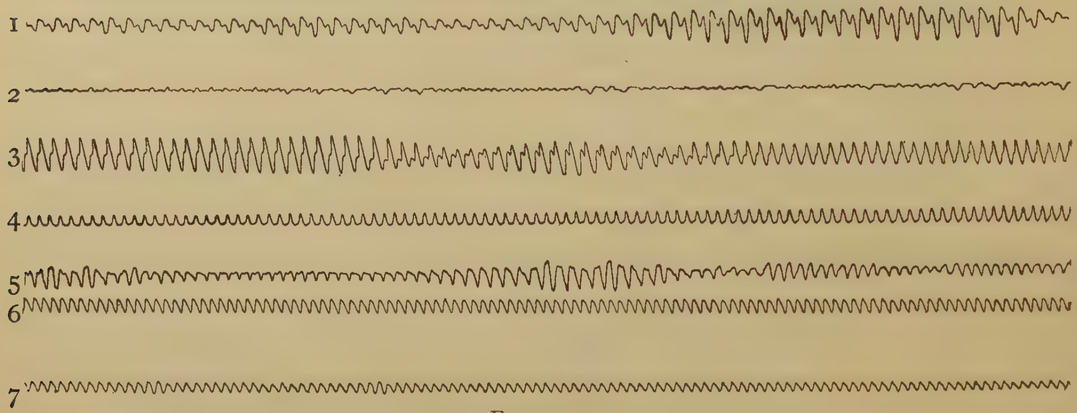

Fig. 2.

For these reasons, a double wave caused by the breaking up of an originally unitary impulse moves slower than did the parent wave. Generally speaking, when a ring is first set into pulsation by electrical stimulation, there are a number of secondary wavelets, but these soon fuse into a single simple wave, and this unitary wave then rarely breaks into component parts.

In *Cassiopea*, as in *Limulus*, the stimulus which produces pulsation is neurogenic, the muscles being incapable of spontaneous pulsation if the nervous system be eliminated. Thus a 0.01 molecular solution of oxalic acid in sea-water is extremely toxic to the motor centers and nerves of *Cassiopea*, a very short immersion preventing the rhopalia from ever again giving rise to the pulsation-stimulus. The muscles, however, still remain capable of contracting when stimulated directly.

In this connection, Harvey found that in most of the amines and in inorganic hydroxides the nerves cease to conduct before the muscles lose their power of contractibility, and under these conditions the muscles respond only when directly stimulated. If the subumbrella tissue be heated, however, the nerves continue to function for several degrees beyond the point whereat the muscles cease to respond by contractions.

The subject of the neurogenic nature of the pulsation-stimulus in *Cassiopea* is discussed by Mayer (1906, p. 19); and, as is well known, Carlson has presented convincing proof that the pulsation-stimulus in the heart of *Limulus* is neurogenic.

The neurogenic nature of pulsation in these invertebrates is the more remarkable because recent work has decidedly strengthened the myogenic theory for the beating of the vertebrate heart. Thus Paton (1907) shows that both the heart and the axial muscular system of *Pristiurus* functions spontaneously before the development of definite nerve-fibrils from the central nervous system, although protoplasmic connectives are seen extending from cell to cell, and these may transmit impulses.

Also, Hooker (1911) finds that the heart differentiates and functions in a normal manner in frog embryos which have developed without a central nervous system; and a crucial experiment has been made by Burrows (1912), who observed that single, isolated muscle-cells of chick hearts, if placed in proper media, pulsate as does the heart itself.

As the experiments of which we are about to speak involve alterations in the composition of the sea-water, it is important that we should understand the composition of the Tortugas sea-water in respect to the cations of sodium, magnesium, calcium, and potassium. Accordingly the following analysis of such of its components as may affect the experiments is presented.

COMPOSITION OF TORTUGAS SEA-WATER.

As a deduction from George Steiger's analysis,[1] made under the direction of Professor F. W. Clarke, it appears that in so far as its ingredients (sodium, magnesium, calcium, and potassium) are concerned, we may make up Tortugas sea-water by mixing the solutions shown under A, each being practically isotonic with the others, and with the sea-water as a whole:

		A.			B.		
81.1	volumes of	0.6006	molecular	NaCl.	0.487	molecular	NaCl.
14.36	" "	0.398	"	$MgCl_2$.	0.0571	"	$MgCl_2$.
2.84	" "	0.389	"	$CaCl_2$.	0.0110	"	$CaCl_2$.
1.7	" "	0.597	"	KCl.	0.0101	"	KCl.
100.00							

Thus any given volume of Tortugas sea-water may be considered to consist of an atmosphere composed as in B, all occupying one and the same space.

At 25° C., however, this being near the usual temperature of the tropical sea-water, the NaCl is dissociated to about 75 per cent; and this is also true of approximately 80 per cent of the $MgCl_2$, 88 per cent of the $CaCl_2$, and 95 per cent of the KCl.

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[1] Carn. Inst., Year Book, No. 9, 1910, p. 122.

Thus among every 4,870 molecules of NaCl, about 1,217 are at any one instant undissociated, while 3,653 are dissociated; or, expressed in tabular form for the sea-water as a whole:

4,870	molecules of	NaCl	=	1,217 fixed	+	3,653 dissociated.
571	"	$MgCl_2$	=	114	+	457
110	"	$CaCl_2$	=	13	+	97
101	"	KCl	=	5	+	96

The NaCl and KCl dissociate each into 2 ions, while the $MgCl_2$ and $CaCl_2$ break up into 3 each, thus:

3,653	dissociated molecules of	NaCl	give	3,653	Nȧ	and	3,653 Cl′.
457	" "	$MgCl_2$		457	Mg̈	"	914 Cl′.
97	" "	$CaCl_2$		97	Cä	"	194 Cl′.
96	" "	KCl		96	K̇	"	96 Cl′.
	Total			4,303			4,857

In other words, in Tortugas sea-water for every 1,000 Nȧ cations there are 125 Mg̈, 26 Cä, 26 K̇; and the anions may be represented by 1,330 Cl′.

As is well known, at least one-fifth of the Cl′ anions may be replaced by the SO_4'' ion without appreciable effect, this being accomplished when the sea-water is made up of sulphates of magnesium, calcium, and potassium instead of chlorides. The cations, on the other hand, play a far more definite rôle in the control of movements; for sustained pulsation is impossible in the absence of even the least concentrated cations, such as Cä or K̇.

If the sea-water be concentrated to 1.69, by evaporating 100 volumes of natural sea-water to 59 volumes, the molecular concentrations of the various constituents becomes:

0.823	molecular	NaCl.
0.0965	"	$MgCl_2$.
0.0186	"	$CaCl_2$.
0.0171	"	KCl.

This results in a relative preponderance of the Mg̈ ions, for the degree of dissociation of the NaCl declines in a greater ratio than that of the $MgCl_2$; for in this concentrated sea-water about 70 per cent of the NaCl is dissociated, as are also about 77 per cent of the $MgCl_2$, 86 per cent of the $CaCl_2$, and 94 per cent of the KCl. Thus:

8,230	molecules of	NaCl	give	2,469	fixed	and	5,761 dissociated.
965	" "	$MgCl_2$	"	222	"	"	743 "
186	" "	$CaCl_2$	"	28	"	"	158 "
171	" "	KCl	"	10	"	"	161 "

And of the dissociated molecules:

5,761	dissociated molecules of	NaCl	give	5,761	Na	and	5,761 Cl′.
743	" " "	$MgCl_2$	"	743	Mg̈	"	1,486 Cl′.
158	" " "	$CaCl_2$	"	158	Cä	"	316 Cl′.
161	" " "	KCl	"	161	K̇	"	161 Cl′.
	Total			6,823			7,724

Hence, we see that when the molecular concentration of the sodium, magnesium, calcium, and potassium is 1.69 times that of natural sea-water, the concentration of the cations of these salts is only $\frac{6823}{4303}$ or 1.585, and, therefore, the concentration of the cations has not quite kept pace with that of the sea-water as a whole.

Moreover, in this concentrated sea-water, for every 1,000 Na cations there are 129 Mg̈, 27 Cä, 28 K̇, and 1,340 Cl′ anions. Thus the proportionate concentration of Nȧ has decreased with respect to the other cations, and as Nȧ is the most potent stimulant while Mg̈ is the depressant, we might expect the stimulating power of this concentrated sea-water to have increased less rapidly than the ratio of concentration, for in natural sea-water the ratio of the Mg̈ to Nȧ is as 1 to 8, and in sea-water concentrated to 1.69 it is as 1 to 7.75.

If, on the other hand, the sea-water be diluted to 50 per cent of its natural concentration by mixing it with an equal volume of distilled water, a calculation, similar to the one given in detail above, indicates that while the molecular concentration of the dissolved salts has declined to 0.5 (that of natural sea-water being 1), the concentration of the cations is 0.528; and for every 100 Nȧ cations there are 124 Mg̈, 26 Cä, and 25 K̇ cations, and 1,376 Cl′ anions. The results of these several calculations are summarized in table 2.

TABLE 2.

Composition of solution. s. w. = sea-water. d. w. = distilled water.	Gram-molecular concentration of the inorganic salts.				Degree of dissociation.			
	NaCl.	$MgCl_2$	$CaCl_2$.	KCl.	NaCl.	$MgCl_2$.	$CaCl_2$.	KCl.
					per cent	per cent	per cent	per cent
100 c.c. s. w. + 100 c.c. of d. w.	0.243	0.0285	0.0055	0.005	79.6	84	92	96
Natural sea-water	.487	.0571	.0110	.0101	75	80	88	95
100 volumes of natural sea-water evaporated to 59 volumes	.823	.0965	.0186	.0171	70	77	86	94

Composition of solution. s. w. = sea-water. d. w. = distilled water.	Approximate number of Mg̈, Cä, and K̇ cations for every 1,000 Na cations.			Relative concentration of cations of sea-water.	Relative concentration of sea-water as a whole.
	Mg̈.	Cä.	K̇.		
100 c.c. s. w. + 100 c.c. of d. w.	124	26	25	0.528	0.5
Natural sea-water	125	26	26	1.000	1.0
100 volumes of natural sea-water evaporated to 59 volumes	129	27	28	1.585	1.69

As the osmotic pressure of these solutions is proportional to the respective sums of their fixed molecules and dissociated ions, we find that if the osmotic pressure of Tortugas sea-water be 1, that of 50 per cent sea-water is 0.5004, and that of 1.69 sea-water is 1.64. Thus, knowing that the osmotic pressure of Tortugas sea-water is about 24.8 atmospheres, that of sea-water diluted with an equal volume of distilled water is 12.4, and that of 1.69 sea-water is 40.7 atmospheres. Table 3 shows the relations between the osmotic pressures and concentrations of the various solutions experimented with in this research, and also the molecular concentration of a solution of NaCl which is practically isotonic with each solution. We must remember, however, that pure NaCl solutions, such as those mentioned in this table, are not, strictly speaking, comparable with those found in sea-water. Thus Osterhout (1911) shows that a pure NaCl solution penetrates the membranes of plant-cells more rapidly than if the NaCl is mixed with $CaCl_2$ in the proportions found in sea-water. There is also other evidence of a chemical combination involving the NaCl and $CaCl_2$ of sea-water in their mutual influence upon animals. For example, Mayer (1906, p. 49) states that in the pulsation of *Cassiopea* the calcium of the sea-water assists the NaCl to counteract the inhibiting influence of magnesium, and later Meltzer and Auer, in an important series of studies upon vertebrates, found that solutions containing *both* calcium and sodium counteracted the inhibiting tendencies of magnesium. Experiments upon *Cassiopea* show that calcium alone can not produce tetanus and does not offset the effects of magnesium if sodium be absent, and thus it appears that the so-called "calcium tetanus" is actually a result of the association of sodium with calcium. Mines (1911 A) believes that calcium enters into combination with cardiac tissue, but magnesium does not, and our results support this view.

Fortunately for physiological experimentation, however, *Cassiopea* is but little affected by considerable changes in osmotic pressure, and table 3 is valid for all practical purposes.

TABLE 3.—*Changes in osmotic pressure and in concentration of dissolved salts in diluted and in concentrated sea-water from Tortugas, Florida.*

Composition of solution. s. w. = sea-water. d. w. = distilled water.	Relative concentration, that of sea-water being 1.	Osmotic pressure in atmospheres.	Gram-molecular concentration of an isotonic solution of pure NaCl.	Gram-molecular concentration of NaCl, $MgCl_2$, $CaCl_2$, and KCl of sea-water solution, regarding sea-water as being a solution of chlorides.			
				NaCl.	$MgCl_2$.	$CaCl_2$.	KCl.
35 c.c. s. w. + 65 c.c. d. w.	0.35	8.7	0.206	0.170	0.02	0.0038	0.0035
40 60	.4	9.9	.236	.2	.023	.0044	.004
50 50	.5	12.4	.295	.243	.028	.0055	.005
58 42	.58	14.4	.342	.285	.033	.0064	.0058
60 40	.6	14.9	.354	.295	.034	.0066	.006
66.6 33.3	.66	16.35	.393	.325	.036	.007	.0066
75 25	.75	18.6	.442	.375	.043	.00825	.0075
Pure sea-water	1	24.8	.59	.487	.0571	.011	.0101
100 c.c. s. w. evap'd to 89.3	1.119	27.4	.66	.545	.064	.0123	.0102
86.8	1.152	28.3	.68	.56	.066	.0126	.0115
84.3	1.186	29.2	.70	.58	.068	.013	.0118
80	1.25	30.6	.737	.61	.071	.0137	.0125
78.1	1.28	31.3	.75	.62	.073	.014	.0128
75.7	1.322	32.2	.78	.64	.075	.0145	.0132
71.1	1.407	34.2	.83	.68	.08	.0154	.014
69.2	1.444	35	.85	.7	.082	.0159	.0147
62.5	1.6	38.6	.94	.78	.091	.0175	.016
59.5	1.68	40.5	.99	.819	.095	.0185	.0168
59	1.69	40.7	1.00	.823	.096	.0186	.0169

RELATION BETWEEN THE CONCENTRATION OF THE CATIONS OF SEA-WATER AND THE RATE OF NERVE-CONDUCTION.

If we dilute sea-water by mixing it with distilled water, or concentrate it by slow evaporation at ordinary temperatures, we not only decrease or increase the concentrations of the electrolytes, but we change the osmotic pressure in a nearly equal ratio. We may, however, avoid lowered osmotic changes by diluting the sea-water with a 0.9 molecular solution of pure dextrose,[1] which is practically isotonic with sea-water, and in this manner we may reduce the concentrations of the electrolytes and at the same time maintain the normal osmotic pressure. Thus we may determine the effect of changes in concentration of the electrolytes independent of osmotic pressure.

The results of such experiments are shown in tables 4, 5, and 6, while table 7 shows the arithmetical averages derived from the results recorded in tables 4 to 6. In all these tables the rate is reduced to 100 per minute in natural sea-water in order to facilitate comparisons. Thus, if on the average the medusæ actually pulsated at the rate of 80 per minute in natural sea-water, and at 40 per minute in some other solution, these numbers would become 100 and 50, respectively, in the tables.

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[1] The dextrose used in these experiments was the best that could be obtained from Merck and from Kahlbaum. The accepted samples were tested for sodium and free acids and found to contain inappreciable quantities of these impurities. The dextrose solutions were made up afresh each day.

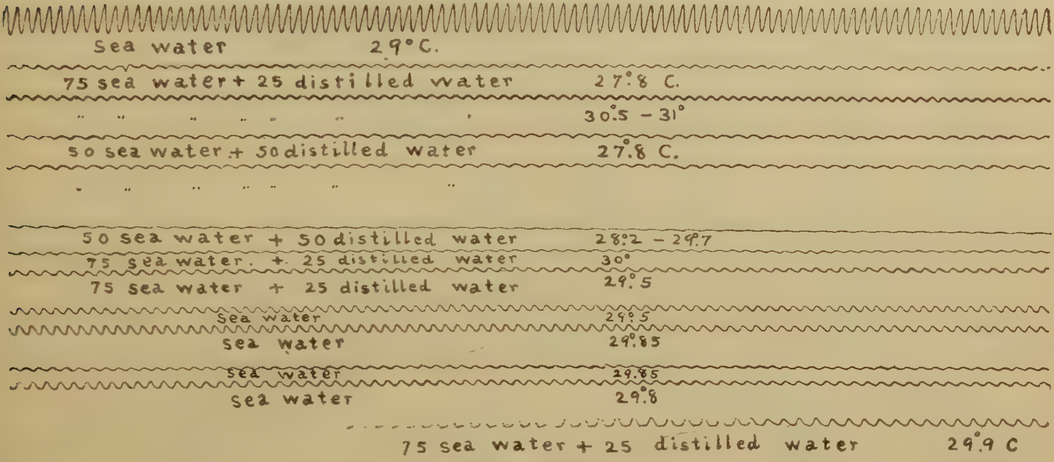

FIG. 3.

Table 4 shows the relative rates of nerve-conduction in 20 rings, and of pulsation in 4 normal medusæ of *Cassiopea xamachana* in sea-water and sea-water diluted with distilled water at 29° to 31° C.

TABLE 4.

Composition of the solution. s. w. = sea-water. d. w. = distilled water.		Osmotic pressure in atmospheres.	Relative rates of pulsation, the rate in normal sea-water being 100.								
			Ring No. 1.[1]	Ring No. 2.	Ring No. 3.	Ring No 4.	Ring No. 5.	Ring No. 6.	Ring No. 7.	Ring No. 8.	Ring No. 9.
Natural sea-water........		24.8	100	100	100	100	100	100	100	100	100
95 p. ct. s. w. +	5 p. ct. d. w.	23.6	......	......	......	......	.....	.....	.....	.....	.....
90	10	22.3	......	......	......	......	.....	.....	.....	.....	.....
85	15	21.8	......	......	......	......	.....	.....	.....	.....	.....
80	20	20.6	......	......	......	......	.....	.....	.....	.....	.....
75	25	18.6	87	86	88	86	81	86	77	86	89
70	30	17.4	......	......	......	......	.....	.....	.....	.....	.....
60	40	14.4	......	......	78	......	65	58	.....	.....	81
50	50	12.4	67	......	64	......	.....	.....	.....	.....	62
40	60	9.9	......	......	......	......	.....	.....	.....	.....	.....
35	65	8.7	......	......	......	......	.....	.....	.....	.....	.....

Composition of the solution. s. w. = sea-water. d. w. = distilled water.		Osmotic pressure in atmospheres.	Relative rates of pulsation, the rate in normal sea-water being 100.								
			Ring No. 10.	Ring No. 11.	Ring No. 12.	Ring No. 13.	Ring No. 14.	Ring No. 15.	Ring No. 16.	Ring No. 17.	Ring No. 18.
Natural sea-water.........		24.8	100	100	100	100	100	100	100	100	100
95 p. ct. s. w. +	5 p. ct. d. w.	23.6	......	......	......	113	.....	105	108	107.5	101
90	10	22.3	......	......	......	121	.....	101	113	106	105
85	15	21.8	......	......	......	104	.....	99	112	107	105
80	20	20.6	......	......	......	111	.....	94	104	96.5	108
75	25	18.6	92	......	94	......	84	.....	.....	.....	.....
70	30	17.4	......	......	......	87	.....	81	83	77	105
60	40	14.4	50	64	......	......	80	72	74.5	69	93
50	50	12.4	......	50	64	......	.....	53	70	61.5	76
40	60	9.9	......	......	......	......	.....	.....	.....	.....	.....
35	65	8.7	......	......	......	......	.....	.....	.....	.....	.....

[1] See record, fig. 3

TABLE 4.—*Continued.*

Composition of the solution. s. w. = sea-water. d. w. = distilled water.	Osmotic pressure in atmospheres.	Relative rates of pulsation, the rate in normal sea-water being 100.							
		Ring No. 19.	Ring No. 20.	General average for the 20 rings.	Normal medusa No. I.	Normal medusa No. II.	Normal medusa No. III.	Normal medusa No. IV.	Normal medusa No. V.
Natural sea-water.........	24.8	100	100	100	100	100	100	100	100
95 p. ct. s. w. + 5 p. ct. d. w.	23.6	103	82	103	......	....	....	....	....
90 10	22.3	100	97	105	......	....	....	....	....
85 15	21.8	89	99	101	......	....	....	....	....
80 20	20.6	84	106	100.5	......	....	....	....	....
75 25	18.6	....	....	86	33	57	....	30	21
70 30	17.4	75	70	82.5	......	....	....	....	....
60 40	14.4	67.5	62	70	......	....	90	30	16
50 50	12.4	53	50.5	61	12	13	60	30	13
40 60	9.9	....	....	....	......	7	40	7	....
35 65	8.7	....	....	....	......	....	28	3	....

TABLE 5.

Composition of the solution. s. w. = sea-water.	Relative rates (rate in pure sea-water being 100).							
	Ring No. 1.[1]	Ring No. 2.	Ring No. 3.	Ring No. 4.	Ring No. 5.	Ring No. 6.	Ring No. 7.	Ring No. 8.
Natural sea-water, salinity 36.15 p. ct.	100	100	100	100	100	100	100	100
95 p. ct. s. w. + 5 p. ct. dextrose.....	....	....	....	....	....	....	....	....
90 10	....	....	....	....	....	....	....	....
85 15	....	....	....	....	....	....	....	....
80 20	....	....	....	....	....	....	....	....
75 25	86	....	86	86	92	98	96	98
71 29	....	....	....	....	....	....	....	....
66.6 33.3	....	....	....	....	....	....	....	....
60 40	....	80	76.5	73	78	73	89	70
58 42	....	....	....	....	....	....	....	....
50 50	55.5	63	....	....	63	....	75	51

Composition of the solution. s. w. = sea-water.	Relative rates (rate in pure sea-water being 100).							
	Ring No. 9.	Ring No. 10.	Ring No. 11.	Ring No. 12.	Ring No. 13.	Ring No. 14.	Ring No. 15.	Ring No. 16.
Natural sea-water, salinity 36.15 p. ct..	100	100	100	100	100	100	100	100
95 p. ct. s. w. + 5 p. ct. dextrose.....	....	101	....	....	....	103	....	....
90 10	....	....	104	105	102	101	....	....
85 15	....	....	....	....	....	....	101	103
80 20	....	98	....	....	....	....	100.5	97.5
75 25	96	95	....	....	....	....	....	....
71 29			82	....	86	92	....	....
66.6 33.3	....	....	....	....	....	....	....	....
60 40	59	79	....	....	....	....	69	....
58 42	....	....	....	....	....	....	....	....
50 50	47	....	55	....	47	....	....	....

Composition of the solution. s. w. = sea-water.	Relative rates (rate in pure sea-water being 100).						
	Ring No. 17.	Ring No. 18.	Ring No. 19.	Ring No. 20.	Gen. av. 20 rings.	Normal medusa No. I.[2]	Normal medusa No. II.[3]
Natural sea-water, salinity 36.15 p. ct.	100	100	100	100	100	100	100
95 p. ct. s. w. + 5 p. ct. dextrose.	....	105	114	96	104	....	....
90 10	....	115	109	....	106	....	....
85 15	103	....	....	108	104	....	....
80 20	99	105	100	....	100	....	....
75 25	....	....	....	....	92.5	47	....
71 29	....	....	....	....	87	....	....
66.6 33.3	....	....	....	....	....	....	23
60 40	....	....	....	66.5	73	....	....
58 42	....	....	....	....	....	....	2
50 50	....	50	57	58	56.5		

[1] See record, fig. 4.

[2] Six hours after being restored to sea-water, rate was nearly normal with amplitude lower than normal

[3] When restored to sea-water, this medusa pulsated at about two-thirds normal rate. See record, fig. 5

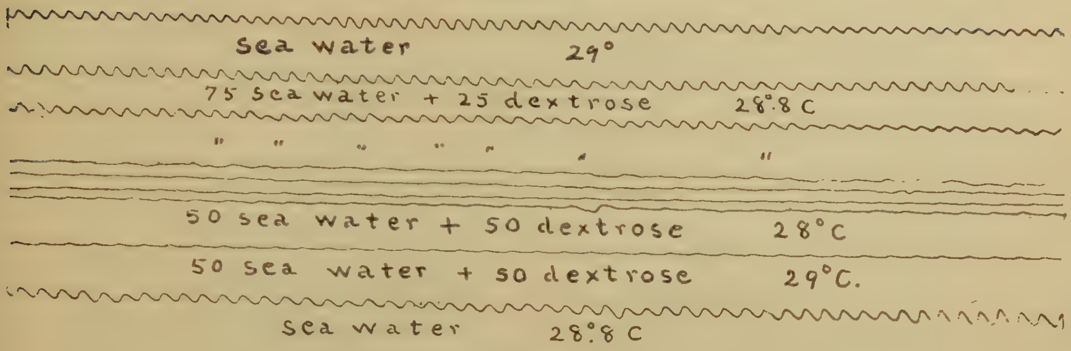

FIG. 4.

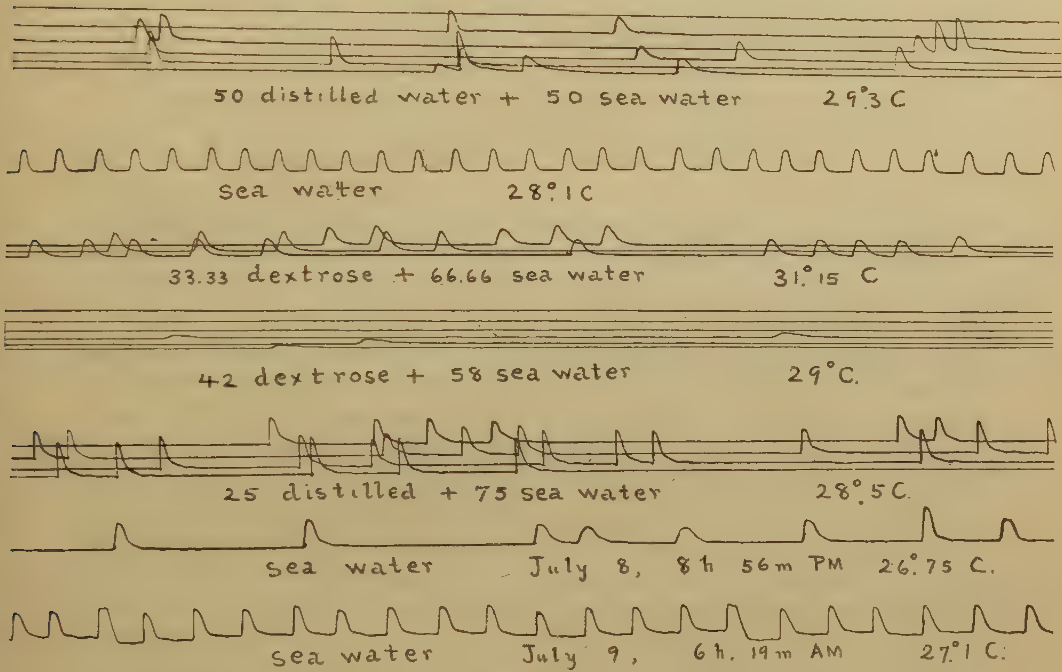

FIG. 5.

Table 5 shows the relative rates of pulsation of 2 medusæ and of nerve-conduction in 20 rings of subumbrella tissue of *Cassiopea* in sea-water, and in sea-water mixed with 0.9 molecular dextrose.

Table 6[1] shows the relative rates of nerve-conduction in subumbrella tissue of *Cassiopea xamachana* in sea-water diluted with distilled water, and also in sea-water diluted with 0.9 molecular dextrose. (See tables 4 and 5.) The practical coincidence of these two curves indicates that the changes in rate of nerve-conduction are due to corresponding changes in the concentration of the electrolytes and not to changes in osmotic pressure.

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[1] See fig. 6, which shows that these two curves are apparently identical, the differences between them being probably due to errors of experimental nature and individual variation.

In figure 6 the full line and open circles represent the rates in sea-water diluted with 0.9 molecular dextrose, and the dotted line and black circles show the rates in sea-water diluted with distilled water.

TABLE 6.

Composition of the solution. s. w. = sea-water. d. w. = distilled water.	Relative rates of nerve-conduction, that in natural sea-water being 100.	
	Sea-water diluted with distilled water, thus correspondingly reducing osmotic pressure.	Sea-water diluted with 0.9 molecular dextrose, thus maintaining normal osmotic pressure of 24.8 atmospheres.
Natural sea-water, salinity 36.15 p. ct.....	100	100
95 p. ct. s. w. + 5 p. ct. d. w. or dextrose...	103	104
90 10 ..	105	106
85 15 ..	101	104
80 20 ..	100.5	100
75 25 ..	86	92.5
71 29 ..	....	87
70 30 ..	82.5	....
60 40 ..	70	73
50 50 ..	50	56.5

Figure 6 gives a graphic representation of the results stated in table 6, the ordinates giving the average rates of the pulsating rings, and the abscissæ giving the relative concentration of the dissolved salts of the sea-water, that of normal sea-water being 100. Thus the ordinates of the full-line curve show the relative rates of conduction of the nerve-impulse when the sea-water is diluted with 0.9 molecular dextrose, and the dotted curve shows the same for sea-water diluted with distilled water so as to reduce its osmotic pressure in nearly the same ratio as the reduced concentration of the electrolytes. The practical coincidence of the curves for sea-water, diluted with distilled water and with 0.9 molecular dextrose, shows that the phenomena in question are not due to variations in osmotic pressure, but to the varying concentrations of the electrolytes.

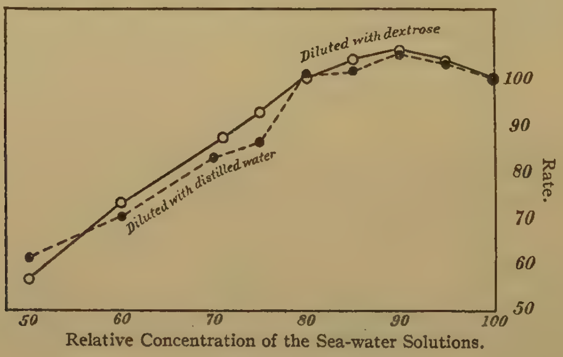

FIG. 6.

Moreover, both these curves show that the most rapid rate of nerve-conduction is in diluted sea-water, wherein the electrolytes are only about

90 per cent as concentrated as in natural sea-water. Below this the rate declines, becoming normal in 80 per cent sea-water and from this point downward it declines in a straight-line ratio, at a somewhat more rapid rate than the corresponding decline in concentration of the dissociated electrolytes.

The curve as a whole reminds one of that of an enzyme reaction having its maximum in 90 per cent sea-water and rising in a straight line from 50 to 80 per cent. This does not *prove*, however, that it has anything to do with enzyme action.

It will be recalled that Loeb (1891) discovered that growth and regeneration in hydroids proceeds at a somewhat more rapid rate in sea-water diluted with 15 to 20 per cent distilled water than in normal sea-water. The experiments of Goldfarb (1907) upon the regeneration and growth rate in hydroids and his more recent observations upon *Cassiopea*[1] confirm this fact. Loeb ascribed the increase in rate of regeneration to the effects of potassium and magnesium, but Goldfarb's observations cast doubt upon this simple explanation. It is clear that the initial increase in the rate of nerve-conduction in *Cassiopea*, which accompanies the decline in concentration of the electrolytes below the normal, is not due to lowered osmotic pressure, for it occurs when the sea-water is diluted with 0.9 molecular dextrose, thus maintaining the osmotic pressure found in normal sea-water.

A possible but purely hypothetical explanation may be that if the rate of nerve-conduction is due to an excess of ionic sodium in the tissues over and above its concentration in the surrounding sea-water, a slight lowering of the concentration of the sodium in the sea-water causes a corresponding increase in its ratio of concentration within the tissues and may thus cause an augmentation in the stimulus. There is some evidence to support this view, for when the sea-water is diluted with 0.487 molecular sodium chloride, the rate does not increase as rapidly as it does when the sea-water is diluted with distilled water or with dextrose. The slight initial increase in rate when the sea-water is diluted with 0.487 molecular NaCl may be due to the reduction of the potassium ion which in such concentration is a stimulant, as is sodium.

THE RELATION BETWEEN THE RATE OF NERVE-CONDUCTION AND THE CONCENTRATION OF THE SODIUM ION.

In order to test the effect of the sodium cation upon the rate of nerve-transmission, we may add increments of a solution of 0.6 molecular NaCl to the sea-water, thus maintaining a constant and normal osmotic pressure, but increasing the concentration of Nä while the concentrations of Mg̈, Cä, and K̇ are decreased.

The physical effects of such a procedure are illustrated in table 7, showing that if we add 0.6 molecular NaCl up to 100 sea-water + 800 NaCl we have changed the concentration of the sodium ion from 1 to 1.205; while at the same time, in comparison with the Mg̈, Cä, and K̇, the Nȧ ion has become relatively 10.85 times as concentrated as it was in natural sea-water.

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[1] See Dr. Goldfarb's paper upon the rate of regeneration of *Cassiopea* in concentrated, normal, and diluted sea-water; page 88 of this volume.

TABLE 7.

Composition of the solution. s. w. = sea-water.	Relative dilution of the Mg̈, Cä, and K̇ of sea-water.	Relative concentration of Nä of sea-water.	Proportionate concentration of Nȧ compared with that of the Mg̈, Cä, and K̇ (proportion in sea-water being 1.00).	Gram-molecular concentrations of—			
				NaCl.	$MgCl_2$.	$CaCl_2$.	KCl.
Natural sea-water.....	1.00	1.00	1.00	0.487	0.057	0.0110	0.0101
100 s. w. + 10 NaCl....	.909	1.008	1.1	.491	.052	.0099	.0091
100 20	.833	1.03	1.23	.506	.047	.00916	.0084
100 30	.7615	1.04	1.4	.51	.043	.00837	.0077
100 40	.714	1.06	1.48	.52	.0407	.00785	.0072
100 50	.666	1.07	1.6	.525	.038	.00739	.0067
100 60	.625	1.08	1.7	.529	.0356	.00687	.0063
100 70	.588	1.09	1.85	.533	.034	.0064	.0059
100 80	.555	1.1	1.98	.537	.0316	.0061	.0055
100 90	.526	1.108	2.1	.54	.03	.0058	.0053
100 100	.50	1.11	2.22	.543	.0285	.0055	.005
100 200	.333	1.15	3.45	.562	.019	.0036	.0033
100 700	.125	1.203	9.62	.586	.007	.0013	.0012
100 800	.111	1.205	10.85	.587	.006	.0012	.0011

Table 8 shows the relative rates of nerve-conduction in four rings of *Cassiopea xamachana* in sea-water to which increments of 0.6 molecular NaCl are added.

TABLE 8.

Composition of the sea-water. s. w. =sea-water.	Relative concentration of Cä, Mg̈, and K̇ of sea-water.	Ring No. 1.	Ring No. 2.	Ring No. 3.	Ring No. 4.	Average condition reduced to 100 pulsations per minute in natural sea-water.
Natural sea-water......	1.00	100	100	100	100	[1]100
100 s. w. + 10 NaCl....	.909	103	....	....	....	103
100 20	.833	102	....	....	....	102
100 30	.7615	105	....	....	....	105
100 40	.714	108	....	....	....	108
100 50	.666	96	....	....	....	96
100 60	.625	97	....	....	....	97
100 70	.588	102	....	....	....	102
100 80	.555	87	....	....	....	87
100 90	.526	82	....	....	....	82
100 100	.50	101	85	....	....	93
100 200	.333	....	90	....	....	90
100 700	.125	....	....	66	87	76.5

[1] See fig. 7, dotted curve with determined points marked by open circles.

Figure 7 shows the stimulating influence of sodium. The dotted curve with determined points marked by open circles represents the effect of 0.6 molecular sodium chloride. The full-line curve shows the less marked effect of 0.487 molecular sodium chloride, and the dextrose curve is introduced for purposes of comparison.

The average results are plotted in the upper dotted line in figure 7 and it appears that the rate of the ring increases slowly in proportion as the sodium becomes concentrated relatively to the other cations, until the relative concentration of the sodium in respect to the other cations is about 1.48 times that found in natural sea-water. At still greater concentration of sodium the rate declines slowly, but not so rapidly as it does in sea-water diluted with a 0.9 molecular solution of dextrose, thus showing the stimulating influence of the sodium ion.

By diluting the sea-water with 0.9 molecular dextrose, we maintain a constant and normal osmotic pressure, but all the cations are reduced in concentration, as they would have been had we added distilled water. On the other hand, when we add 0.6 molecular NaCl we increase the absolute concentration of the sodium, while the osmotic pressure of the solutions as a whole remains constant, but the chief effect is to increase the relative concentration of the sodium cation with respect to magnesium, calcium, and potassium.

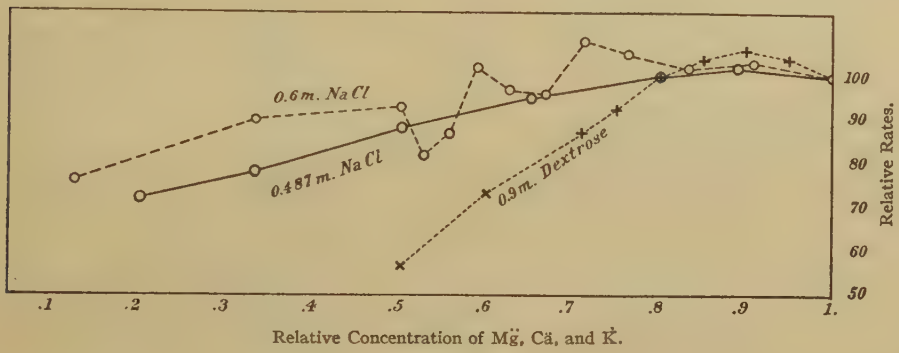

FIG. 7.

We may conclude that sodium is a powerful stimulant up to about 1.48 times its relative concentration with respect to the other cations in natural sea-water augmenting the rate of nerve-conduction in proportion as its relative concentration increases. In concentrations over and above 1.48 times that found in sea-water, it loses some of its stimulating effect, but the rate of pulsation of the ring declines more slowly than it would in sea-water diluted with a 0.9 molecular solution of dextrose, and thus the presence of the sodium still acts as a constant stimulant.

The experiment cited above is open to the serious objection that the addition of 0.6 molecular NaCl increases the concentration of the Nȧ ion over and above that found in natural sea-water, and the stimulating effect is evidently due in part at least to this increase, and not to the proportionate decrease in concentration of magnesium, calcium, and potassium. In order to avoid this error, we should add the NaCl in the form of a 0.487 molecular solution dissolved in a 0.075 molecular solution of dextrose, thus maintaining the normal osmotic pressure and the normal concentration of the sodium ion—*simply reducing* the concentrations of the magnesium, calcium, and potassium, and correspondingly increasing the relative concentration of the sodium cation.

Table 9 shows the results of this experiment upon 20 subumbrella rings of *Cassiopea*, and here again it is evident that the sodium cation is a stimulant to nerve-conduction. It maintains the rate of nerve-conduction at about a normal rate until the magnesium, calcium, and potassium are reduced to 80 per cent of the concentration found in natural sea-water.

In still greater dilutions there is a slow decline in rate in a rectilinear ratio, but the decline is not so rapid as it is when all four cations, sodium, magnesium, calcium, and potassium, are reduced by adding 0.9 molecular dextrose to the sea-water. Thus the sodium of the normal sea-water is a stimulant to the rate of nerve-conduction.

As we would expect, the 0.6 molecular NaCl is more stimulating than the lower concentration, 0.487 molecular.

TABLE 9.

Composition of solution. s. w. = sea-water.	Relative rates of pulsation, the rate in normal sea-water being 100.						
	Ring No. 1.	Ring No. 2.	Ring No. 3.	Ring No. 4.	Ring No. 5.	Ring No. 6.	Ring No. 7.
Sea-water (salinity 36.15 p. ct., osmotic pressure 24.8 atmospheres)	100	100	100	100	100	100	100
89 p. ct. s. w. + 11 p. ct. (NaCl + dextrose)	....	....	....	....	....	....	103
80 20	....	....	....	....	102	98	101
65 35	....	....	....	....	....	....	....
50 50	87.5	86	85.5	97	82	84	....
33.3 66.6	....	....	77.5	78	74	....	....
20 80	....	62.5	65.5	85.5	....	....	70.5

Composition of solution. s. w. = sea-water	Relative rates of pulsation, the rate in normal sea-water being 100.						
	Ring No. 8.	Ring No. 9.	Ring No. 10.	Ring No. 11.	Ring No. 12.	Ring No. 13.	Ring No. 14.
Sea-water (salinity 36.15 p. ct., osmotic pressure 24.8 atmospheres)	100	100	100	100	100	100	100
89 p. ct. s. w. + 11 p. ct. (NaCl + dextrose)	99	113	99	98	....	....	....
80 20	90.5	120	92	86	102.5	106	102
65 35	....	....	....	....	....	....	....
50 50	....	....	....	....	95	....	90
33.3 66.6	84	78	....	....	....	....	....
20 80	....	....	....	....	....	....	....

Composition of solution. s. w. = sea-water.	Relative rates of pulsation, the rate in normal sea-water being 100.						
	Ring No. 15.	Ring No. 16.	Ring No. 17.	Ring No. 18.	Ring No. 19.	Ring No. 20.	Gen. av.
Sea-water (salinity 36.15 p. ct., osmotic pressure 24.8 atmospheres)	100	100	100	100	100	100	[1]100
89 p. ct. s. w. + 11 p. ct. (NaCl + dextrose)	....	....	....	....	....	....	102
80 20	....	....	....	....	....	....	100
65 35	100	81	96	103.5	....	....	95
50 50	....	....	....	....	....	....	88
33.3 66.6	78	....	....	....	68.5	75	78
20 80	....	....	....	....	....	....	72

[1] See fig. 7, full-line curve.

Table 9 shows the relative rates of nerve-conduction in 20 subumbrella rings of *Cassiopea xamachana* in sea-water and in sea-water diluted with a solution composed of 0.487 molecular NaCl dissolved in 0.075 molecular dextrose. This solution maintains the normal concentration of NaCl and the normal osmotic pressure of Tortugas sea-water and its addition merely causes a decline in the concentrations of the magnesium, calcium, and potassium of the sea-water.

As in all other experiments of this series, the perfect medusæ are more sensitive to the effects of sodium than are the rings, but the difference in

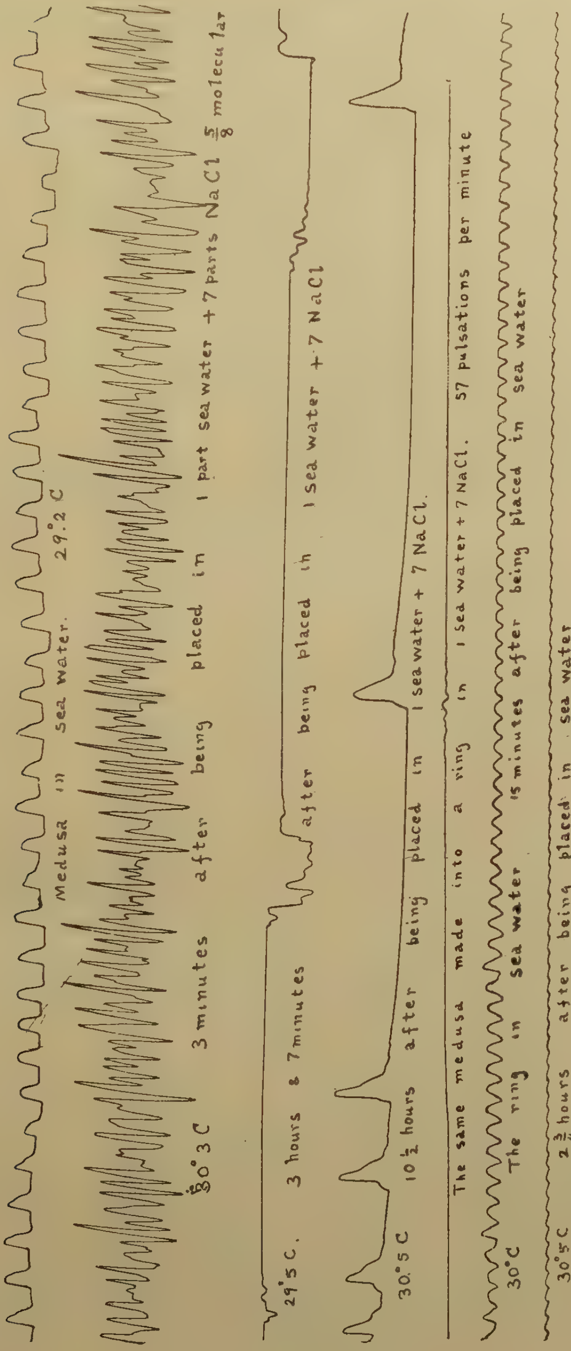
Medusa in sea water. 29°.2 C
30°3 C 3 minutes after being placed in 1 part sea water + 7 parts NaCl 5/8 molecular
29°5 C. 3 hours & 7 minutes after being placed in 1 sea water + 7 NaCl
30°5 C 10½ hours after being placed in 1 sea water + 7 NaCl.
The same medusa made into a ring in 1 sea water + 7 NaCl. 57 pulsations per minute
30°C The ring in sea water 15 minutes after being placed in sea water
30°5 C 2¾ hours after being placed in sea water

FIG. 8.

their behavior is one of degree rather than of kind. In other words, the motor centers are merely a more sensitive part of the nervous system than is the nerve-net of the subumbrella.

For example, a medusa of *Cassiopea xamachana* (record, fig. 8) with motor centers intact, and pulsating at the rate of 33 per minute in natural sea-water, pulsated very irregularly at the rate of about 8 per minute after having been for 10.5 hours in a solution of 100 volumes of sea-water + 700 volumes of 0.6 molecular NaCl. Here we see the decline in pulsation-rate is greater than in the nervous network without motor centers. If, however, the sea-water had been diluted with 0.9 dextrose, the medusa would have ceased to pulsate when the sea-water had been mixed with about an equal volume of dextrose; hence the effect of the lowering of concentration of Mg̈, Cä, and K̇ and proportionately increasing the concentration of the sodium ion is to stimulate and maintain pulsation.

The initial effect of an increased concentration of NaCl upon the normal medusa is to increase its rate of pulsation. Thus the medusa which pulsated at the rate of 33 per minute in sea-water, immediately pulsated at the rate of about 116 per minute when placed in 1 volume of sea-water plus 7 volumes of 0.6 molecular NaCl, although after half an hour its rate was about normal and after 10.5 hours it was only about 8 per minute. Thus the initial effect of the relatively increased concentration of sodium was to stimulate, but later it produced depression in a manner resembling the effect of its close chemical ally, potassium. It is interesting to see that Magowan (1908) and Osterhout (1909) find that sodium and potassium are closely similar both in their toxic and protective action upon plants.[1]

THE INERT NATURE OF THE MAGNESIUM ION.

In order to determine the effects of the magnesium ion, we may add magnesium to sea-water in the form of a 0.057 molecular solution of $MgCl_2$ dissolved in 0.78 molecular dextrose. This decreases the concentration of the sodium, calcium, and potassium cations, but maintains the normal concentration of the magnesium ion and also the normal osmotic pressure of the sea-water. The effect must, therefore, be due to the decline in concentration of the sodium, calcium, and potassium.

Table 10 and figure 9 (dotted curve with open circles) show the results of this experiment upon 7 rings of subumbrella tissue of *Cassiopea*, and it indicates that the magnesium ion, in the concentration found in sea-water, is not in any sense a stimulant; for the rate of nerve-conduction declines in practically the same manner is it does when the sea-water is diluted with distilled water or with 0.9 molecular dextrose.

The non-toxic nature of magnesium is clearly shown by the good and rapid recovery of the rate of pulsation when the rings are replaced in natural sea-water.

Figure 9 shows the effects of magnesium on the rate of nerve-conduction. The full-line curve shows the effect of adding 0.4 molecular $MgCl_2$ to sea-water. The dotted curve applies to 0.057 molecular $MgCl_2$. The crosses show the effects of adding 0.9 molecular dextrose to sea-water, and the black circles show the effects of increments of distilled water added to sea-water.

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[1] Botanical Gazette, vol. 45, p. 45; vol. 48, p. 98.

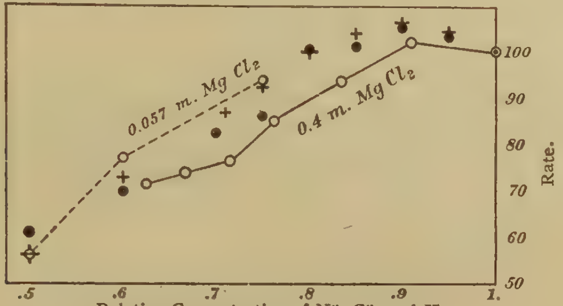

FIG. 9.

Table 10 shows the decline in rate of nerve-conduction in 7 subumbrella rings of *Cassiopea* in sea-water diluted with a solution composed of 0.057 molecular $MgCl_2$ + 0.78 molecular dextrose.

TABLE 10.

Composition of the solution. s. w. = sea-water.	Relative rate of nerve-conduction, the rate in normal sea-water being 100.							
	Ring No. 1.	Ring No. 2.	Ring No. 3.	Ring No. 4.	Ring No. 5.	Ring No. 6.	Ring No. 7.	Gen. av.
Natural sea-water	100	100	100	100	100	100	100	100
90 p.ct. s. w. + 10 p.ct. $MgCl_2$ + dextrose[1]	......	......	......	......	......	......	......	......
75 25	97	99	91	97	100	92	90	95
60 40	79	80	82	89	80	79	54	77.5
50 50	......	71	......	56	49.5	55	47.5	56
Rate an hour after being returned to normal sea-water	......	91	......	......	93	99	......	......

[1] Only two rings were tested in this solution and they both gave relative rates of 94. This rate is probably too low to be typical, and therefore I have rejected the results obtained from these two rings and omitted them from the table.

By another method, which was tried at Tortugas in 1911 and 1912, the normal osmotic pressure was maintained by adding to the sea-water a 0.4 molecular solution of $MgCl_2$ in distilled water. This causes a dilution of

TABLE 11.

Composition of the solution. n. s. w. = natural sea-water.	Gram-molecular concentration of NaCl, $MgCl_2$, $CaCl_2$, and KCl.				Relative concentration of the Nȧ, Cä, and K̇, their concentration in natural sea-water being 1.	Relative concentration of the Mg̈, their concentration in natural sea-water being 1.	Proportionate concentration of Mg̈ compared with that of Nȧ, Cä, and K̇, the proportion in natural sea-water being 1.
	NaCl.	$MgCl_2$.	CaCl.	KCl.			
Natural sea-water	0.487	0.0571	0.0110	0.0101	1.000	1.000	1.0
100 c.c. n. s. w. + 10 c.c. $MgCl_2$	.443	.0883	.00999	.00918	.909	1.546	1.7
100 20	.406	.1142	.00916	.00841	.8333	2	2.4
100 30	.3745	.1372	.00837	.0077	.7615	2.402	3.15
100 40	.348	.1550	.00785	.0072	.714	2.714	3.801
100 50	.325	.1713	.00739	.0067	.666	3	4.504
100 60	.304	.1875	.00687	.00631	.625	3.284	5.25

the sodium, calcium, and potassium ions and an augmentation in the concentration of the magnesium, as is shown in table 11.

Table 12 gives the decline in rate of pulsation shown by 2 rings and 9 normal medusæ of *Cassiopea xamachana* in sea-water to which increments of 0.4 molecular $MgCl_2$ have been added.

TABLE 12.

Composition of the solution. s. w. = sea-water.	Relative concentrations of Na, Ca, and K.	Relative rates of pulsation.					
		Ring No. 1.[1]	Ring No. 2.[2]	Medusa No. 3.[3]	Av. of medusæ Nos. 4 and 5.[4]	Av. of medusæ Nos. 6, 7, and 8.[5]	Av. of medusæ Nos. 9, 10, and 11.[6]
Natural sea-water............	1.00	[7]100	100	[8]100	100	100	100
100 c.c. s. w. + 2.5 c.c. $MgCl_2$..	.97	....	....	....	....	....	65.5
100 5 ..	.95	....	....	....	62	70	80
100 7.5 ..	.93	....	....	....	....	....	63
100 10 ..	.909	107	97.5	51	28	51	46
100 12.5 ..	.888	....	....	....	....	....	31
100 15 ..	.871	....	....	....	23	35	19
100 17.5 ..	.85	....	....	....	....	....	21
100 20 ..	.833	94	94	23	15	23	14.15
100 22.5 ..	.817	....	....	....	....	....	6
100 25 ..	.8	....	....	....	11	1	6
100 27.5 ..	.78	....	....	....	....	....	1
100 30 ..	.761	88.5	82	....	0	0	....
100 40 ..	.714	75	78	....	....	....	....
100 50 ..	.666	68	80	....	....	....	....
100 60 ..	.625	71	72	0	....	....	....

[1] 15.5 hours after being replaced in sea-water this ring pulsated at the normal rate.
[2] 3 hours after being replaced in sea-water this ring pulsated at its normal rate.
[3] After being replaced in sea-water this medusa recovered perfectly and pulsated at its normal rate.
[4] After being replaced in sea-water they soon recovered and pulsated normally.
[5] These medusæ recovered completely and pulsated normally after being replaced in sea-water.
[6] Upon replacing these medusæ in sea-water, they pulsated at normal rates.
[7] A partial record of this ring is given in record, fig. 19.
[8] See record, fig. 11.

Table 13, which is derived from table 12, shows the average rates of nerve-conduction in two rings of *Cassiopea xamachana* in sea-water and in sea-water mixed with 0.4 molecular $MgCl_2$.

TABLE 13.

Composition of the solution. s. w. = sea-water.	Av. rate per min. of the two rings.	Av. rate reduced to 100 per min. in normal sea-water.	Dilution of the Na, Ca, and K of the sea-water.	Relative concentration of Mg, its concentration in sea-water being 1.
Natural sea-water..............	141.5	[1]100	1.0	1.0
100 s. w. + 10 $MgCl_2$............	144.5	102	.909	1.7
100 20	133	94	.833	2.4
100 30	120	85	.7615	3.15
100 40	108	76.5	.714	3.8
100 50	104	74	.666	4.5
100 60	100.5	71.5	.625	5.25

[1] See fig. 9, full-line curve.

Table 14 shows the average rates of pulsation of 9 medusæ of *Cassiopea xamachana* in sea-water and in sea-water mixed with 0.4 molecular $MgCl_2$. The rates are reduced to a scale of 100 in natural sea-water.

Tables 12 and 13 show the results of experiments upon two pulsating rings in sea-water at 28° to 31° C. to which had been added increments of 0.4 molecular $MgCl_2$ dissolved in distilled water. The rings or medusæ were permitted to pulsate in any new concentration of magnesium for at least 1 and usually 3 hours before a record of the pulsation-rate was taken, and in the interval the solution was changed several times. In this manner an attempt was made to permit the animal to become thoroughly permeated by a solution before its effect was tested.

TABLE 14.

Composition of the solution. s. w. = sea-water.	Relative rate reduced to 100 in normal sea-water.	Dilution of concentration of $\dot{Na}$, $\ddot{Ca}$, and $\dot{K}$ of the sea-water.	Relative concentration of Mg (concentration in sea-water being 1).
Natural sea-water............	100	1.000	1.0
100 c.c. s. w. + 10 $MgCl_2$.......	44	.909	1.7
100 20	17.5	.8333	2.4
100 25	2.5	.8	2.2
100 30	0	.7615	3.15

Tables 13 and 14 show the average conditions; table 13 referring to the rate of nerve-conduction in the rings without motor centers, and table 14 to the pulsation of medusæ with motor centers intact. It is evident that any excess of magnesium causes a rapid decline in rate in the perfect medusæ.

In rings of subumbrella tissue 0.4 molecular $MgCl_2$ causes a decline in rate of nerve-conduction which is but little more pronounced than that observed when the sea-water is diluted with distilled water or with 0.9 molecular dextrose.

This leads one to conclude that the magnesium ion is inert rather than an active repressor of nerve-conduction. If this be the case all concentrations of $MgCl_2$ from 0.4 molecular downward should behave alike and should exert the same influence as distilled water or 0.9 molecular dextrose.

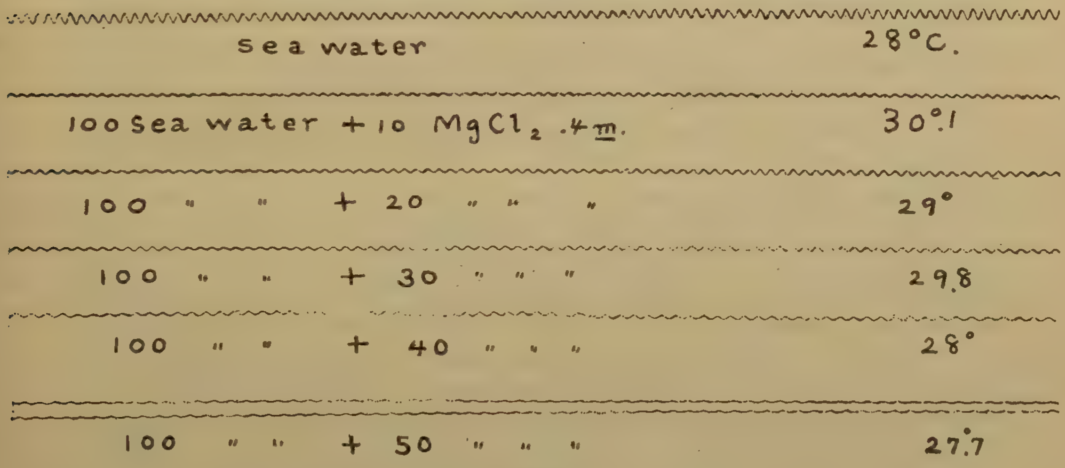

FIG. 10.

Figure 9 appears to show that this expectation is largely realized, the effects of 0.057 molecular and of 0.4 molecular $MgCl_2$ being closely similar to those of distilled water, and while 0.4 molecular $MgCl_2$ may exert a slight specific depressant effect, it is evident that the concentration of $\ddot{Mg}$ found in sea-water (0.057 molecular) is not more depressant than is distilled water.

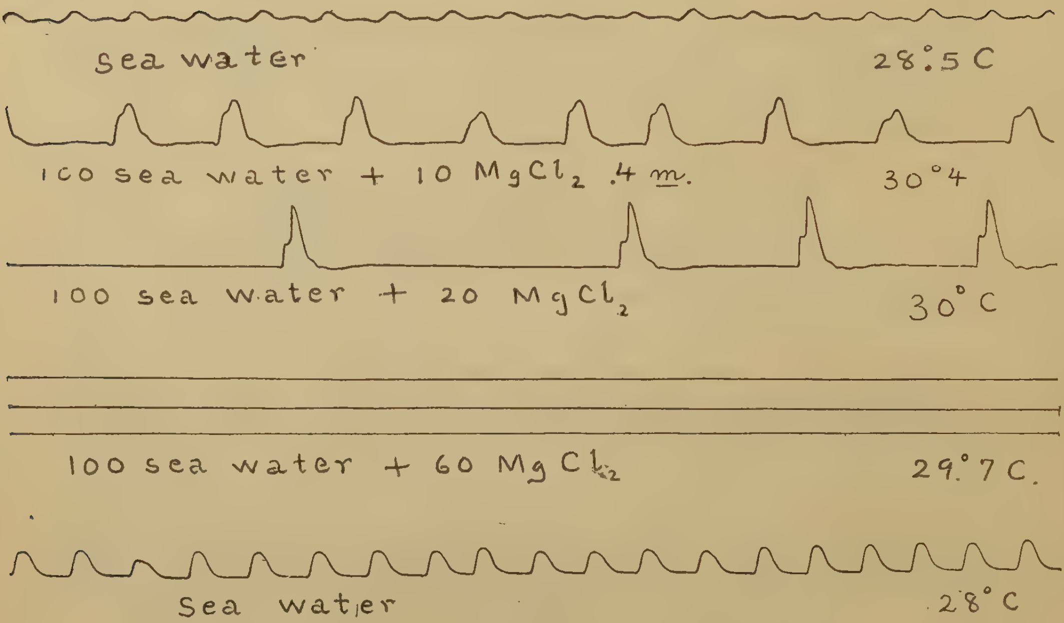

FIG. 11.

Thus apart from osmotic effects the degree of concentration of magnesium makes but little difference in stupefying marine animals by Tullberg's method of adding $MgCl_2$ or $MgSO_4$ to the sea-water. The comparative unimportance of the magnesium ion may also account for the fact that while the relative concentrations of the sodium, calcium, and potassium in the blood of mammals remain pretty much as they are in sea-water, the magnesium has declined from 3.7 to 0.4 or to about one-ninth its former concentration (see "The fitness of the environment," p. 187, by Laurence J. Henderson, New York, 1913).

Normal medusæ soon cease to pulsate if placed in a solution of 100 c.c. sea-water + 30 c.c. of 0.4 molecular $MgCl_2$, and they may remain in this solution for 8 days without further pulsation, the bell, however, slowly disintegrating, due to bacterial action which the weakened concentration of sodium is unable to check. Whenever these magnesiumized medusæ are replaced in normal sea-water they at once go into clonic tetanus and then pulsate normally. This initial tetanus resembles that which develops in sea-water lacking magnesium, and is there due to the combination of sodium and calcium unchecked by magnesium. It seems, then, that an excess of magnesium acts as a shield or "buffer" to prevent the calcium from producing tetanus, and when this shield is suddenly removed the medusa is unable to withstand the effects of even the normal concentration of calcium.[1] It will be recalled that Mines (1911 B, p. 185) arrested the pulsation of the heart of a ray by magnesium and then restored pulsation by simply raising the hydrogen ion concentration of the solution from about 6.5 to 9. Thus the inhibiting effect of the $\ddot{Mg}$ was offset by the addition of an alkali.

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[1] Sörensen, 1909, Comptes Rendus Lab. Carlsberg, vol. 8, p. 1, calls attention to the action of various sorts of "buffer" substances in physiological solutions.

It appears from my experiments of 1906 that calcium can not produce tetanus unless sodium be present, for in the absence of sodium even pure solutions of 0.4 molecular $CaCl_2$ do not produce tetanus in *Cassiopea*, and if the medusæ be stupefied in magnesium they can not be temporarily revived by calcium unless sodium be present. These observations are in accord with those of Meltzer and Auer on mammals, wherein they found that the inhibiting tendency of magnesium is offset by a calcium-sodium combination, not by calcium alone.

As in all experiments of this series, normal medusæ are much more sensitive to the effects of magnesium than are rings. In other words, as is always the case, the motor centers are more readily affected than are the peripheral nerves.

EFFECTS OF THE POTASSIUM ION.

We may maintain the normal osmotic pressure and increase the concentration of the potassium ion by adding to the sea-water a 0.64 molecular solution of KCl. Even so small an amount as 3.75 c.c. of this solution added to 100 c.c. of sea-water stops all movement in normal medusæ after an initial excitement which soon subsides. The peripheral nerves, on the other hand, are not so readily affected as the motor centers, for a ring still pulsated at about two-thirds its normal rate when placed in 100 c.c. sea-water + 7.5 c.c. of 0.64 molecular KCl.

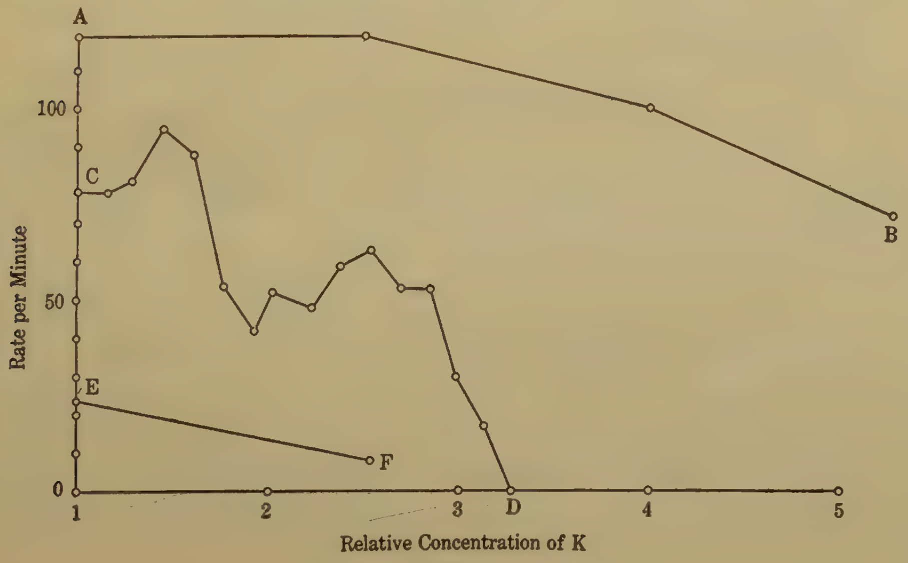

FIG. 12.

Table 15 and figure 12 illustrate the results of experiments with potassium. The rates stated in figure 12 are the actual rates assumed by animals after being at least 45 minutes in the solutions mentioned, the volume of solution being very great in comparison with the size of the animals and the solutions being changed frequently.

It appears that a very slight increase over and above the normal concentration of the potassium ion is a permanent stimulus.

Table 15 shows the relative rates of 3 normal medusæ and 1 subumbrella ring of *Cassiopea xamachana* in sea-water to which increments of a 0.64 molecular solution of KCl have been added.

TABLE 15.

Composition of the solution. s. w. = sea-water.	Relative concentration of potassium cation, its concentration in natural sea-water being 1.	Relative rate of 3 normal medusæ.[1]	Relative rate of a normal medusa.[2]	Relative rate of a ring of subumbrella tissue.
Natural sea-water	1.00	[3]100	[4]100	[5]100
100 c.c. s. w. + 0.25 c.c. KCl	1.16	100		
100 0.5	1.28	103		
100 0.75	1.44	122		
100 1.	1.6	112		
100 1.25	1.76	69		
100 1.5	1.92	54		
100 1.75	2.07	67		
100 2.	2.22	61.5		
100 2.25	2.37	75		
100 2.5	2.53	81	33	101
100 2.75	2.69	67		
100 3.	2.84	67		
100 3.25	2.98	37		
100 3.5	3.13	22		
100 3.75	2.27			
100 5.	4.			85
100 7.5	5.3			62

[1] These medusæ recovered their normal rates after being returned to sea-water.

[2] Stopped in less than one hour. This medusa recovered its normal pulsation when returned to sea-water.

[3] See curve CD, fig. 12.

[4] See curve AB, fig. 12, and record, fig. 13.

[5] See curve EF, fig. 12.

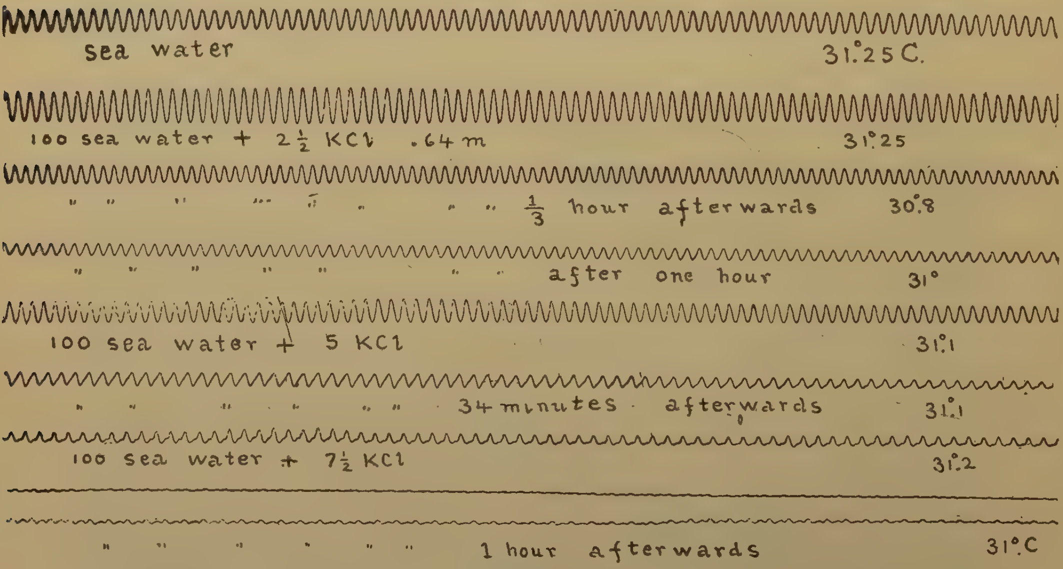

FIG. 13.

If we place normal medusæ in a solution having the proportions of 100 c.c. sea-water + 1 c.c. of 0.64 molecular KCl, their rates are increased and maintained thus for more than 24 hours. This solution gives the potassium ion a concentration 1.6 times as great as in natural sea-water. Any further increase in the concentration of the potassium ion causes an initial excitement, but this is soon followed by a loss in rate. Rings are not so sensitive and the nerves may maintain a normal rate in a solution having 2.5 times the concentration of the potassium ion in sea-water, but a still further concentration causes a decline in rate after temporary, initial excitement.

It has long been known that weak concentrations of potassium cause initial excitement followed by loss of rate. It is, however, interesting to see that a very weak concentration may be a permanent stimulant, for it shows that potassium acts in a manner similar to that of sodium, its close associate in Group I of the periodic system. Like potassium, sodium itself produces sustained increase in rate in weak excess, but depression in stronger concentrations. Also in stronger concentrations its initial effect is to stimulate, but depression soon follows. Sodium also is slightly toxic, potassium even more so. Thus in all these respects potassium resembles sodium, but its metallic properties are more decided, as indeed we would expect from its higher atomic weight.

EFFECTS OF CALCIUM.

It is difficult to determine the effects of the calcium ion *per se*, for (as is well known) it associates itself or combines with the sodium ion, possibly forming a sodium-potassium ion proteid, and it enables the sodium to counteract the negative effect of magnesium.

If *Cassiopea* be placed in a van't Hoff's solution resembling sea-water, but lacking calcium, all movement soon ceases, but pulsation begins to be revived when we add a concentration of calcium equal to 0.4 or 0.5 that found in natural sea-water. Pulsation is most rapid, however, when the concentration of the calcium ion is about 2.5 times that found in natural sea-water. Further additions of calcium cause increasing depression and movements usually cease when the solution contains 7 times the concentration of calcium found in sea-water. Such a solution is not toxic, however, for complete recovery takes place almost immediately if the medusæ be restored to sea-water.

It is interesting to see that half the normal concentration of calcium suffices to restore pulsation, for half the normal concentration of sodium will accomplish the same end, provided magnesium, calcium, and potassium be present in normal concentration.

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III.

THE LAW GOVERNING THE LOSS OF WEIGHT IN STARVING CASSIOPEA.

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One plate and twenty-one figures.

THE LAW GOVERNING THE LOSS OF WEIGHT IN STARVING CASSIOPEA.

By Alfred Goldsborough Mayer.

METHODS.

Before being weighed each medusa was dried upon filter paper so as to remove the water adherent to its surface. It was then weighed in 25 c.c. of sea-water on a delicate balance, the weight of the medusa being determined by subtracting the weight of the water and the glass beaker serving as its container.

The medusæ were starved in sea-water which had been passed through two glass funnels, each containing a double layer of the best quality of Chardin's filter paper, thus removing the zoöplankton upon which *Cassiopea* feeds. In some of the experiments the sea-water after having been passed through Chardin's filters was refiltered through porcelain or it was rendered sterile in so far as animal life was concerned by heating it to 71° C. and then restoring the evaporation by distilled water. These additional precautions were, however, found to be unnecessary for the Chardin filters removed all or practically all food from the water.

The medusæ were always starved in the purest sea-water which was either dipped from the ocean in glass or canvas buckets or pumped into glass reservoir tanks through hard-rubber pipes by means of a hard-rubber pump. In running comparative series care was taken that the sea-water in which each medusa was starved had had the same history; *i. e.*, was gathered in the same way, stored in the same tank, etc., for every medusa in the series. Whenever possible the medusæ were starved side by side in one and the same glass aquarium, but when this was impossible the aquaria were of similar size and form and were placed side by side, so as to be subjected to similar environmental changes.

EXPERIMENTS.

It is a pleasure to acknowledge my indebtedness to Dr. Francis Gano Benedict for important advice and highly appreciated kindness, and to Mr. Joseph C. Bock, of the Nutrition Laboratory of the Carnegie Institution of Washington, for having determined the nitrogen content of the medusæ.

In common with other scyphomedusæ, *Cassiopea xamachana* consists of a large central mass of gelatinous substance, which is covered with a thin layer of cellular tissue, the weight of the gelatinous substance being very great in proportion to that of the cellular elements of the medusa's body.

When the animal starves, this gelatinous substance decreases in volume and apparently serves as the chief store of food for the starving animal.

If W be the weight of the medusa when starving begins, aW may represent the decline in weight due to loss of body-substance and of water at the end of the first day, so that at the end of the first day the weight of the medusa is $W - aW = W(1 - a)$.

Similarly, at the end of the second day the weight is $W(1 - a) - aW(1 - a) = W(1 - a)^2$.

Hence the weight y after starving x days is $y = W(1 - a)^x$ where a may be called the index of catabolism, the rate of starvation increasing as a increases.

This formula accords fairly well with the facts of observation, as will be apparent from an inspection of tables 4 to 27 and the curves accompanying them. At times the observed weights are above and at other times below the theoretical curve, but the general average in table 1 for 30 normal medusæ starved in the diffuse daylight of the laboratory is practically coincident with the curve $y = W(1 - 0.567)^x$ for a period of 19 days starving in filtered sea-water.

Table 1 and figure 1 show the average decline in weight deduced from six series of experiments upon 30 medusæ of *Cassiopea xamachana* starved 19 days *in diffuse daylight* in doubly filtered sea-water. The weights are reduced to a scale of 100 grams at the beginning of the experiment: Thus, if a medusa weighed 10 grams at the beginning and 5 grams at the end of a certain number of days, these weights would appear as 100 and as 50, respectively, in the accompanying table:

TABLE 1.

Days elapsed.	Observed average weight.	Calculated weight.[1]
0	100	100
2	88.5	88.97
4	77.82	79.16
6	71.35	70.43
9	59.73	59.11
12	50.44	49.61
15	42.08	41.64
19	32.9	32.9

[1] Calculated according to the formula $y = 100(1 — 0.0567)^x$.

This simple law governing the loss of weight in *Cassiopea* leads to the conjecture that the chemical constitution of the animal does not change, but that one and the same class of substances serves to maintain the medusa throughout the period of its starvation. It is well known that in vertebrates a very different condition pertains, for the starving animal at first mainly consumes its store of glycogen, oxidizing it into carbohydrate; then the fats are chiefly drawn upon, so that a starving vertebrate contains relatively less fat and more proteid and water than a normal creature. Thus there is a progressive change in the chemical constitution of the body of the animal.

This appears not to be the case in *Cassiopea*, and this supposition receives further support from an analysis of the percentage of nitrogen in the body, which appears to range from 2.121 to 2.997 per cent of the dried weight of the animal, the variation being independent of the number of days the animal has starved. Hence it seems probable that starving neither increases nor decreases the percentage of nitrogen. The results are shown in table 2, wherein it appears that there is no coördination between the number of days the medusa has been starved and the percentage of nitrogen it contains. The medusæ were desiccated at 100° C. at Tortugas, Florida, and the dried substance was then sent to Boston for analysis.

TABLE 2.

Days starved.	Live weight in grams just before being killed.	Dried weight in grams.	Per cent of dried to living weight.	Per cent of nitrogen to dried weight.
0	109.055	5.401	4.9	2.566
0	109.641	5.43	4.9	2.147
3	91.9	4.425	4.8	2.476
4	157.71	6.91	4.3	2.48
6	....[1]	5.972	....	2.997
10	94.380	4.38	4.6	2.121
12	86.72	4.362	5.0	2.226
14	40.288	1.93	4.8	2.451
16	83.232	3.981	4.8	2.543
			Av. 4.762	Av. 2.445

[1] Not weighed.

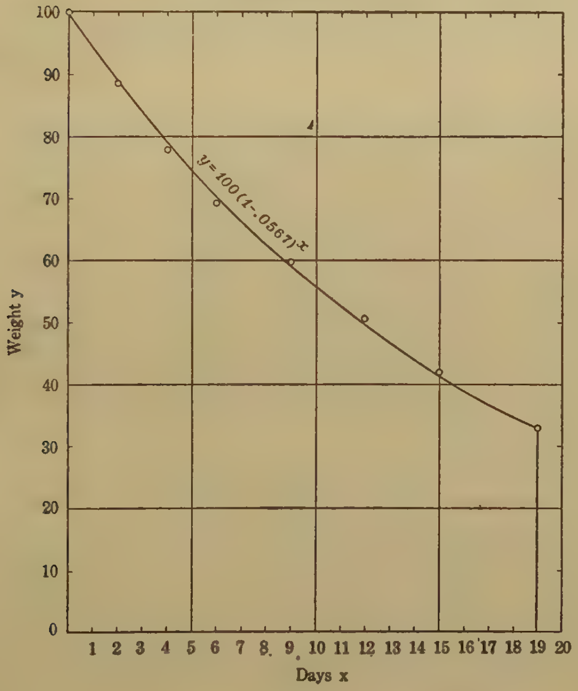

FIG. 1.

It appears also that the dried weight is about 4.76 per cent[1] of the living weight, and that this ratio does not change as the animal starves. Thus the living weight bears a practically constant ratio to the weight of the solid substance of the animal, and the law governing the loss of rate may be determined either from the one or the other, the living weight being preferable on account of its greater magnitude and the consequent lessening of the degree of error in its determination. Hence the reason for the validity of the simple law that governs the loss of weight in the starving jellyfish is that no appreciable chemical change occurs in the composition of its body-substances, and there is no appreciable selective use of different substances at different times during the progress of starvation.

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[1] Of this 4.7 per cent, 3.5 per cent consists of the salts of sea-water; and thus the actual body-substance of the medusa is only 1.2 per cent, the animal containing 98.8 per cent of water.

Sections show that as a physical result of starvation, the cells become reduced in size, many degenerate and disappear, and the cell boundaries tend to become indistinct; moreover, the gelatinous substance becomes vacuolated and the muscular tonus is largely lost, so that the bell-rim bends upward and inward in a balloon-like manner, as is shown in plate 1, figure A, which represents 6 medusæ that have been starved 41 days and whose combined weights have declined from 74.95 to 2.78 gm., all being still alive. In fact, throughout these series of experiments no medusa has been lost through natural death, although some of the series of observations upon medusæ starved in the dark were terminated on account of the development of local maceration due possibly to ineffectually checked bacterial activity.

Plate 1, figure B, gives an enlarged view of the smallest of these medusæ shown in plate 1, figure A, and one may observe its crumpled, shrunken bell, the loss through coalescence of the mouths, and the absorption of the mouth-arm appendages. Indeed, the first organs to disappear are usually the mouths and their appendages, and thus after being starved for about 3 weeks recovery may become impossible through the inability of the animal to capture the zoöplankton upon which the medusa feeds. Experiments indicate that they do not feed upon diatoms, but in common with the corals, as determined by Vaughan, their food appears to be exclusively of an animal nature, the smaller forms of the zoöplankton being taken into the numerous mouths on the mouth-arms.

It is known that much of the nannoplankton can pass through the best filters, but one was unable to discover any specimens of the zoöplankton in the doubly filtered sea-water used in these experiments. Nevertheless, medusæ with their stomachs removed starve more rapidly than do animals with their stomachs intact but regenerating their bell-rims. The medusæ which possess stomachs are, however, not called upon to regenerate any of their parts and are thus subjected to less drain upon their resources.

The value of a in the formula $y = W(1 - a)^x$ gives us a fair measure of the rate of loss of weight. It may be called the "coefficient of negative metabolism," for as a increases the loss of weight increases in like ratio.

In the experiments recorded in this paper a ranges as shown in table 3.

It appears from table 3 that *Cassiopea* thrives best and starves at its slowest rate if maintained in large quantities of sea-water changed only once in each 24 hours, thus resembling the conditions of the semistagnant lagoons in which the medusa lives.

TABLE 3.

Medium in which medusæ were starved and conditions of the experiment.	Value of a when medusæ are starved in diffuse daylight of laboratory.	Value of a when medusæ are starved in dark.	Tables referred to.
Normal medusæ each in 6 liters or more of doubly filtered sea-water changed once in every 24 hours.	0.046 to 0.075; av. 0.0567..	0.0639 to 0.075; av. 0.0695	4 to 10 and 14
Medusæ regenerating bell-rim but with stomach and mouth-arms intact, each in 6 liters or more of doubly filtered water changed once in each 24 hours.	0.0681 to 0.0689; av. 0.0685	0.0511 to 0.0538; av. 0.052	19 to 25
Regenerating disks with stomach and mouth-arms removed, each in 6 liters or more of doubly filtered water changed once in each 24 hours.	0.06 to 0.1034; av. 0.0815..	0.074 to 0.0937; av. 0.0892	18 to 25
Normal medusæ in 400 c.c. of doubly filtered water changed once in each 24 hours.	0.1145..................	0.1...................	11 and 12
Normal medusæ in large quantities of doubly filtered constantly changing sea-water.	0.1015 to 0.15; av. 0.125...	0.165 to 0.185; av. 0.175..	15 and 16
Normal medusa in 6 liters of sea-water sterilized by heating it to 71° C., and then cooling it to normal temperatures. The water being changed once in each 24 hours.	0.055..................	....	17

The relative rates of starvation both in darkness and in diffuse daylight, shown by medusæ under other conditions, are as follows, the results being derived from table 3.

Normal medusæ in large amounts of stagnant water	1.0
Medusæ regenerating their bell rims, but with stomach and mouth-arms intact	0.96
Medusæ starving without stomachs	1.27
Normal medusæ in small aquaria of 400 c.c. capacity	1.7
Normal medusæ in constantly running water	2.4
Normal medusæ in water sterilized by heating it to 71° C.	1.0

Although for the first week or more the regenerating medusæ generally lose weight more rapidly than do the normal animals the final rate of starvation appears to be practically the same in normal and in regenerating medusæ provided their stomachs be present, and this is in accord with the well-known converse fact that the rate of regeneration is independent of the food-supply, starving animals regenerating quite as rapidly as well-fed ones.

In this connection it appears from an inspection of the curves accompanying tables 4 to 27, that if the starving medusæ lose but little weight for a day or two, a succeeding period of abnormally rapid loss is apt to ensue and thus the animals tend to follow the theoretical curve $y = W(1 - a)^x$.

Cassiopea lives in shallow lagoons of semistagnant water, where it lies fully exposed to the hot sun and, as table 3 shows, it loses weight slowly when kept in quantities of water of about 6 liters per animal in aquaria the water of which is changed only once in each 24 hours. If the water be changed constantly, however, or the medusæ be kept in running water, they starve more rapidly than if maintained in stagnant water.

It will be recalled that Allen and Nelson[1] found that if sea-water be heated to 70° C. practically all life within it is killed, and the water remains sterile unless reinfected.

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[1] Allen, E. J., and E. W. Nelson (1910). Quarterly Journal of Microscopical Science, London, vol. 55, pp. 361–431; also, Journal of Marine Biology Association, vol. 8.

Bearing this in mind, the sea-water was doubly filtered and then heated to 71° C. after which it was allowed to cool in sterilized glass vessels protected from dust. Medusæ kept in this sterilized water, whether in the dark or in daylight, lost weight no more rapidly than would have been the case had they been starved in sea-water that had been merely passed through four Chardin filter papers without being sterilized by heating.

When sea-water is heated to 71° C., however, some of its dissolved air is expelled, and in order fully to compensate for this medusæ were starved in a control sea-water solution which had been placed in a vacuum under the receiver of an air pump, thus expelling far more air than was lost by the sea-water which had been heated to 71° C. It was found, however, that the commensal plant-cells of the medusa render the animal comparatively independent of the oxygen of the sea-water, provided an excess of CO_2 be absent, and thus the medusa starves at practically the same rate in heat-sterilized, air-exhausted, or normal filtered sea-water. In fact, it starves at this same rate in sea-water which has been doubly filtered through the Chardin filters and then refiltered through porcelain to extract bacteria and nannoplankton. Putter's[1] "dissolved food" of sea-water may, therefore, still be available as food even after all organisms have been taken out of the sea-water, and it appears that such organisms are not necessary to initiate a process of assimilation.

If the stomach be intact, the animals starve less rapidly than if it be removed. Animals whose stomachs have been cut off can regenerate new gastro-vascular centers, but this is rarely done, and it so happened that none of the disks studied by me succeeded in replacing their stomachs, although all made more or less progress in regeneration.

In order to determine the effect of the regeneration, we compared the starving rate of these agastric medusæ with others having stomachs intact but regenerating their bell-rims, an annulus of the bell being cut off close to the outer edge of the stomach, as is shown in plate 1, figure C.

Table 3 and tables 18 to 25 show that disks without stomachs lose weight about 1.25 times as rapidly as do regenerating medusæ with stomachs intact.

Carbon dioxide plays a conspicuous rôle in controlling the activities of the medusa, for Harvey[2] found that during the day the velocity of nerve-conduction is greater than during the night, and this fact is possibly due to the variation in the supply of oxygen conditioned by symbiotic algæ in the subumbrella tissue of the medusa.

It was found that if we make a weak solution of rosolic acid in sea-water and divide it into two equal volumes in two separate containers and then cut a medusa into halves and place each half in a volume of the rosolic-acid solution, it will be observed that if one half of the medusa be kept in diffuse daylight and the other half in darkness, the rosolic acid surrounding the darkened half of the medusa loses color and fades rapidly, while the fluid surrounding the half of the medusa which is in daylight fades more slowly. This and the lime-water test in magnesium-free sea-water show that the medusa gives off CO_2, and that in diffuse daylight less CO_2 is given off than in darkness; the difference being apparently due to photosynthesis on the part of the commensal plant-cells or zoöxanthellæ which infest the gelatinous substance immediately under the external cell-layers of the medusa.

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[1] The most complete presentation of Putter's views is given in his recent work "Die Ernährung der Wasserthiere und der Stoffansalt der gewasser" (Jena, 1910).

[2] Harvey, E. Newton. Carnegie Institution of Washington, Year Book No. 10, 1911, p. 131.

Tables 3, 11, and 12 show that when the medusæ are confined in a small volume of water they starve more rapidly than if kept in large aquaria. They also pulsate slower when confined in small aquaria, and a slight excess of CO_2 brings them to rest, but even when not moving they lose weight far more rapidly than if pulsating in normal sea-water, the toxic effect of CO_2 being very noticeable. If a few bubbles of CO_2 be passed through sea-water the medusæ cease to pulsate; plate 1, figure A, is derived from a photograph of animals treated in this manner. Upon replacing the medusæ in normal sea-water pulsation is immediately renewed, no permanent toxic effects appearing until they have been subjected to the CO_2 for about 24 hours.

The influence of CO_2 is probably counteracted by the well-known photosynthesis due to the commensal plant-cells or zoöxanthellæ.

When the medusæ are starved in diffuse daylight these plant-cells become more and more crowded as the medusa declines in bulk, and after several days of starving they begin to escape early in the morning, when the swarming zoöxanthellæ penetrate the ectoderm and pass out into the ocean, where they swim actively, and finally settle upon those lighted parts of the cylindrical glass aquarium which are away from the source of light, the swarming cells being entrapped in the lighted meniscus of the aquarium in accordance with the Jennings reaction. When, on the other hand, the medusæ are starved in the dark, the algal cells are rendered inactive and do not escape into the water. Medusæ starved in the light become dark brown in color after the first 10 days, for the plant-cells remain alive, although apparently unhealthy and probably inactive.

When the medusæ are starved in darkness many of the plant-cells die and dissolve *in situ* in the gelatinous substance. Not all of these cells die, however, for a few still remain alive and apparently healthy after being more than a month in the dark, and if the medusa be replaced in the light these cells propagate and again provide the animal with a normal supply of zoöxanthellæ. Sections show that when the medusæ starve in the dark the gelatinous substance becomes vacuolated and edemic, due possibly to the unreduced excess of CO_2 in the tissues. Thus the medusæ when starved in the dark appear to suffer from lack of oxygen, being in effect partially suffocated. Yet even when the plant-cells have been greatly reduced through 26 days of starvation in the dark, the medusæ can still pulsate for 2 or more hours at nearly a normal rate in sea-water that has been boiled to expel its oxygen. It at one time seemed that these plant-cells played a significant rôle in maintaining the weight of the medusa when starved in daylight, but further experiments have shown that this is not the case, for the medusæ sometimes starve at a more rapid rate in diffuse daylight than in the dark, although the reverse is usually the case.

It is not the object of this paper to present an exhaustive review of all previous researches upon the metabolism of inanition in invertebrates, because previous studies do not touch upon the chief subject of this study—the law of the decline of weight. A brief mention of certain important sources of general information should, however, be presented.

The earliest work appears to be that of Dumas and Milne-Edwards (1820, Annales Chim. et Physique, tome 14; and also in the Comptes Rendus, Paris, 1843, tome 17, p. 531) upon the metabolism of bees.

Peligot (1865, Comptes Rendus, tome 61, p. 866; and 1867, Annales Chim. et Physique, tome 12, p. 445) studied silkworms in the same connection.

Especially worthy of mention is the work of Slowtzoff[1] (1904), who found that when starved the vineyard slug consumed about 25.74 per cent of its total weight and about 28.41 per cent of its store of energy. The relation between the organic and inorganic substance remains the same in the normal and in the starved animals. The loss of vital substances consists of fat, carbohydrates, and water in the proportions of carbohydrate 93.98 per cent, fat 78.51 per cent, and water about 30.02 per cent, while the loss of albumen was computed to be 23.70 per cent. As a result of starvation the soft parts of the animal acquire an increase of insoluble salts to about 35.9 per cent. During the period of starvation the slugs consume 4.84 calories of energy per kilogram of their weight per 24 hours. The phosphoric albumens were largely retained, only about 19 per cent of these being parted with.

In 1901 the same author[2] starved the dung beetle, *Geotrypes stercorarius* and found that in from 5 to 11 days it lost about 21 per cent of its original weight, the loss appearing to be chiefly water and fat.

Schulz[3] starved *Planaria lactea* and states that after 6 months without food some of the worms were reduced to about one-tenth their original size. There were, however, considerable individual differences, some of the animals being three times as large as the smallest after all had starved for 6 months. Schulz made a histological study of the starved worms and found that some of the cells were killed as a result of the starvation, while others had degenerated or become reduced in size. The epithelium tends to fuse and its cells to degenerate into a syncytium, very much as we have observed in *Cassiopea.*

Perkins[4] observed that the cells of the attached hydroid larvæ of *Gonionemus murbachii* degenerated in aquaria, where presumably they were unfed or poorly nourished, and that the larvæ became amœboid and changed into creeping plasmodia without cell boundaries. Thacher[1] also observed a process of degeneration in hydroid colonies of *Eudendrium*, *Pennaria*, and *Campanularia* kept in aquaria. The polypites of these forms are absorbed, the degenerating cells of the entoderm and finally the ectoderm of the hydranths being turned into the digestive tract of the stem.

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[1] Slowtzoff, B. (1904). Beitrage zur vergleichenden Physiologie der Hungerstoffwechsels, Mittheil. 2, Der Hungerstoffwechsel der Weimbergschnecke (Hofmeister's Beitrage).

[2] Slowtzoff, B. (1909). *Idem.*, Mittheil 5, Der Hungerstoffwechsel der Mistkäfer (*Geotrypes stercorarius*). Biochemischer Zeitschrift, Bd. 19, p. 504.

[3] Schulz, E. (1904). Ueber Reductionen, I, Ueber Hungerscheinungen bei *Planaria lactea.* Archiv für Entwicklungsmechanik der Organismen, Bd. 18, pp. 555–577, Taf. 34, 11 fign.

[4] Perkins, H. F. (1902). Proc. Acad. Natural Sci., Philadelphia, vol. 54, p. 765; also: 1902. Biol. Bulletin Woods Hole, vol. 3, pp. 172–180.

By contrast with the few studies upon invertebrates many researches have been conducted upon the metabolism of matter and energy in the body of starving vertebrates. To properly review this literature would carry us too far afield and indeed a text-book would be required for the purpose, but the literature of the subject, in so far as it relates to man and the higher vertebrates, has been exhaustively reviewed by Atwater and Langworthy (1897) in their "Digest of Metabolism Experiments" (434 pp., Bulletin No. 45, United States Department of Agriculture, Office of Experiment Stations). One should also consult such later works upon vertebrates as Atwater and Benedict (1903), Benedict (1907), Weber (1902), Ergebnisse der Physiologie; Schaefer (1898), Text Book of Physiology, vol. 1, p. 891; Manca (1902), Pembrey and Spriggs (1904), Abderhalden, Bergell, and Dorpinghaus (1904), Hatai (1904), Cathcart and Fawsitt (1907), and Maignon (1908), and others referred to by these authors, the list of papers cited by Pembrey and Spriggs in the Journal of Physiology (Cambridge, vol. 31, pp. 320–345), and by Benedict (1907), being especially enlightening.

EXPLANATION OF THE TABLES.

In the figures accompanying tables 4 to 25, when the medusæ are starved in darkness their observed weights are indicated by black dots; when starved in the diffuse light of the laboratory their weights are shown by circles.

Tables 4 to 25 refer to *Cassiopea xamachana* and table 26 to the allied species *C. frondosa*, which follows a law in its starvation similar to that of *C. xamachana*.

Table 4 shows the decline in weight of 6 normal medusæ of *Cassiopea xamachana* starved 41 days in filtered sea-water, in the *diffuse light* of the laboratory, at temperatures ranging from 27.3° to 30.1° C., from June 18 to July 29, 1911, at Tortugas, Florida. The medusæ were kept in a 6-liter glass aquarium, the water being changed once in every 24 hours. Compare this with table 10 to see the effect of darkness upon medusæ subjected to conditions as nearly as possible identical with the medusæ starved in daylight. The curve $y = 75(1 - 0.075)^x$ does not accord well with the facts, but the curve represented by $y = 75(1 - 0.058)^x$ accords fairly well with the observed decline in weight up to the sixteenth day, after which it departs more and more widely from the observed weights and is unsatisfactory. (See table 5.)

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[1] Thacher, H. F. (1903). Biol. Bulletin Woods Hole, vol. 4, pp. 96–98.

TABLE 4.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	74.95	75
1	73.36	....
2	68.315	64.13
3	65.345	....
4	62.975	54.9
5	59.065	....
6	55.37	46.95
7	51.62	....
8	47.955	40.2
9	45.16	....
10	42.69	34.35
11	40.225	....
12	36.28	29.4
13	33.825	....
14	31.185	25.2
15	29.11	....
16	28.065	21.52
17	27.77	....
18	24.78	18.45
19	23.15	....
20	21.44	15.75
21	18.95	....
22	18.18	13.50
23	17.01	....
24	15.67	11.55
25	14.46	....
26	12.98	9.90
27	11.73	....
28	10.37	8.47
29	9.21	....
30	8.03	7.23
31	7.26	....
32	6.63	6.18
33	6.04	....
34	5.34	5.29
35	4.84	....
36	4.46	4.53
37	4.05	....
38	3.67	3.87
39	3.39	....
40	3.07	3.31
41	2.78	3.07

[1] According to formula $y = 75(1 - 0.075)^x$.

TABLE 5.

Days elapsed.	Observed weights.	Calculated weight.[1]
0	74.95	75
5	59.065	55.65
10	42.69	41.7
15	29.11	30.6
20	21.44	22.72
25	14.46	16.8
30	8.03	12.45
35	4.84	9.22
40	3.07	6.87

[1] According to formula $y = 75(1 - 0.058)^x$.

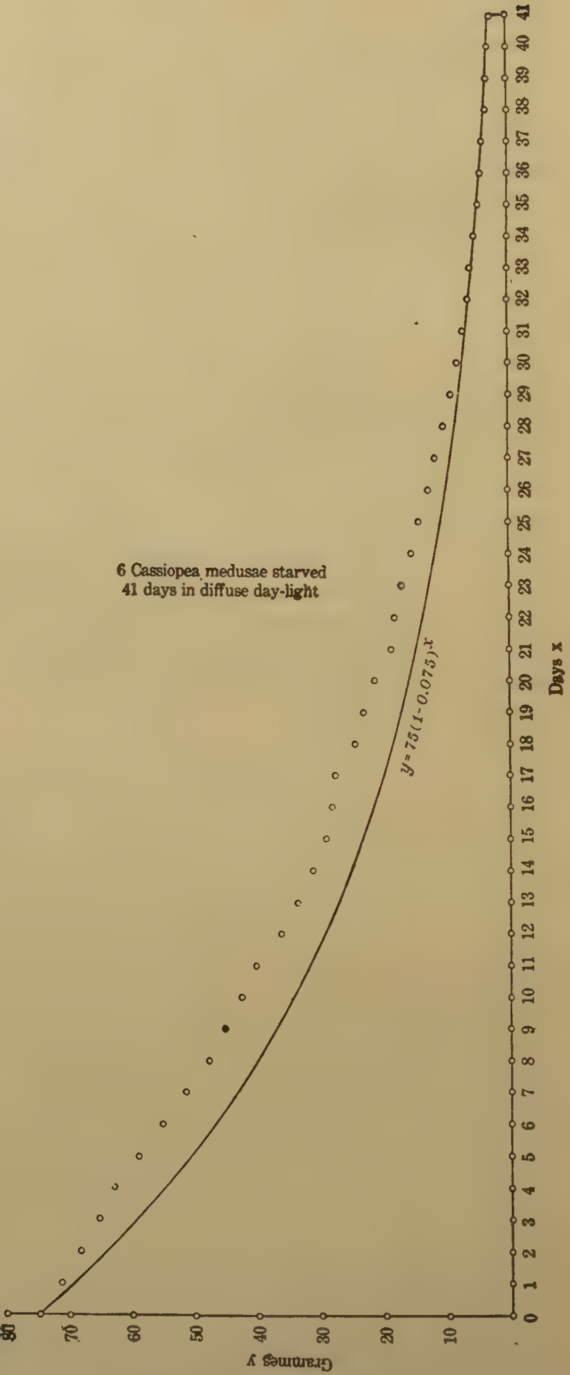

Diagram illustrating Table 4.

Table 6 shows the decline in weight of 10 normal medusæ of *Cassiopea xamachana* starved 25 days in filtered sea-water in the *diffuse light* of the laboratory from June 22 to July 17, 1910, at Tortugas, Florida. The medusæ were kept in a 6-liter glass aquarium, the water being changed once in each 24 hours.

TABLE 6.

Days elapsed.	Observed weight, in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	130.75	131	9	92.14	85.67	18	56.79	56.07
1	128.96	124.97	10	88.71	81.74	19	53.45	53.58
2	119.58	119.21	11	86.24	78.08	20	51.81	51.09
3	115.34	113.71	12	79.54	74.41	21	48.20	48.73
4	108.97	108.6	13	72.31	71	22	45.84	46.5
5	106.58	103.49	14	69.27	67.73	23	44.71	44.41
6	103.55	98.77	15	66.81	64.58	24	42.78	42.31
7	97.60	94.19	16	....[2]	61.7	25	39.7	40.35
8	97.22	89.87	17	61.24	58.82	....	....	....

[1] According to formula $y = 131(1 - 0.046)^x$.

[2] No weighing.

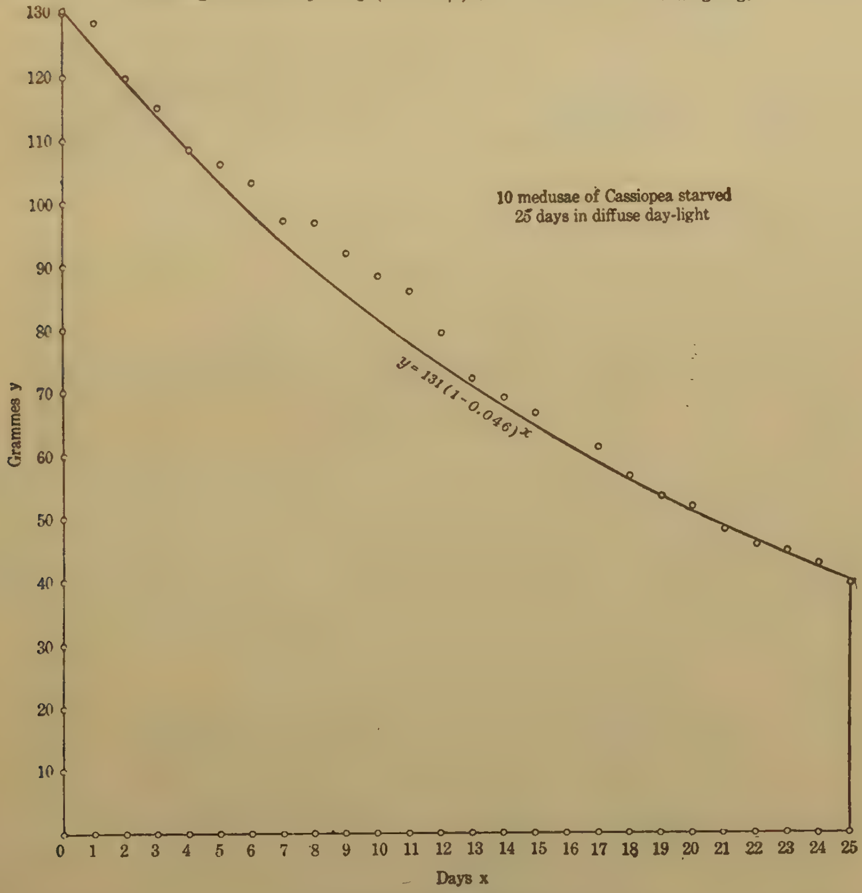

Diagram illustrating Table 6.

Table 7 shows the decline in weight of 11 normal medusæ of *Cassiopea xamachana* starved 22 days in filtered sea-water in the *diffuse light* of the laboratory, at temperatures ranging from 27.1° to 30.1° C., from June 19 to July 11, 1911, at Tortugas, Florida. The medusæ were kept in a 6-liter glass aquarium, the water being changed once in each 24 hours.

TABLE 7.

Days elapsed.	Observed weight in grams.	Calculated. weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	496.9	497	8	328	321.56	16	223.2	208.24
1	457	470.66	9	315	304.66	17	206.9	197.31
2	432.1	445.8	10	306	288.86	18	191.2	186.87
3	399.69	421.95	11	290.99	273.35	19	179.2	176.93
4	385.1	399.59	12	278.95	258.94	20	172.2	167.49
5	369.3	377.22	13	266.3	245.02	21	161.63	158.54
6	365.5	358.83	14	249.5	232.10	22	150.09	150.25
7	345	339.45	15	240.8	219.67	....	....	....

[1] According to formula $y = 497(1 - 0.0529)^x$.

REMARKS.—Free-swimming algal cells were given off from these medusæ every morning soon after daylight for the first week. These plant-cells collected on the side of the cylindrical glass aquarium which was away from the light.

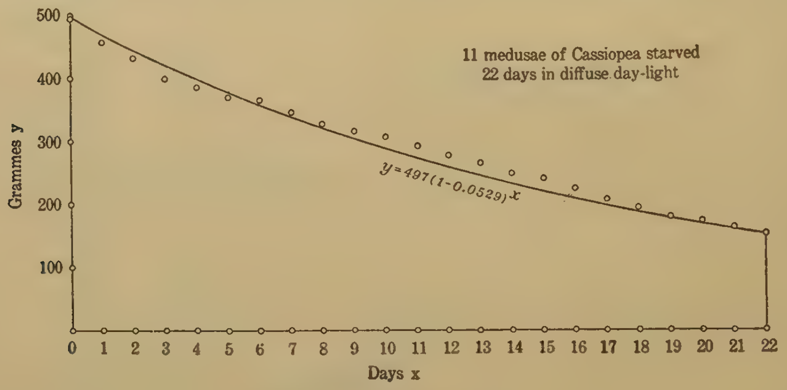

Diagram illustrating Table 7.

Table 8 shows the decline in weight of a single normal medusa of *Cassiopea xamachana* starved 17 days in the *diffuse daylight* of the laboratory in filtered sea-water at temperatures ranging from 26.5° to 30.1° C., from June 22 to July 9, 1911, at Tortugas, Florida. The medusa was kept in a 6-liter glass aquarium, the water being changed once in each 24 hours.

TABLE 8.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	261.95	262	10	133.1	132.31
1	248.5	244.7	11	128	123.66
2	243.9	288.46	12	121.95	115.28
3	231.5	213.53	13	112	108.21
4	215.8	199.38	14	110.2	102.61
5	192.75	186.28	15	101.8	94.06
6	185.7	173.97	16	93.2	87.77
7	166.7	162.44	17	81.9	82
8	158.3	151.7			
9	144.7	141.74			

[1] According to the formula $y = 262(1 - 0.66)^x$.

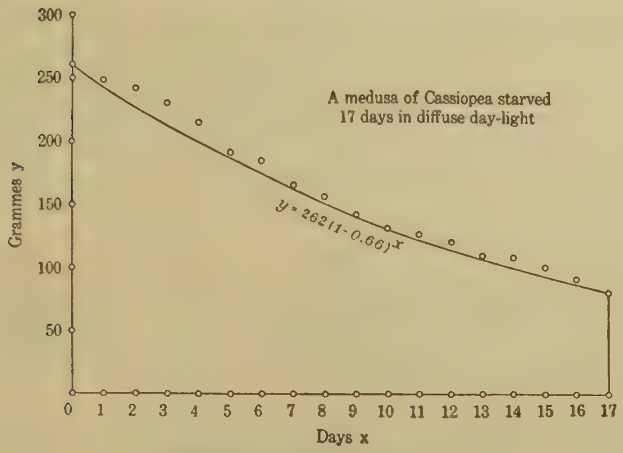

Diagram illustrating Table 8.

Table 9 shows the decline in weight of two normal medusæ of *Cassiopea xamachana* starved 25 days in *diffuse daylight*, in filtered sea-water, at 27.5° to 30° C., from June 28 to July 23, 1912, at Tortugas, Florida. The medusæ were placed in a glass aquarium holding 6 liters, the water being changed once in each 24 hours.

TABLE 9.

Days elapsed.	Medusa *A*.		Medusa *B*.	
	Observed weight in grams.	Weight.[1]	Observed weight in grams.	Weight.[2]
0	42.62	42.62	54.9	54.9
4	31.73	32.00	36.48	43.2
7	26.94	25.76	34.94	36.18
10	20.52	20.80	29.08	30.19
13	16.48	16.79	25.51	25.25
17	13.25	12.57	19.41	19.87
21	10.19	9.46	14.99	15.70
25	7	7	12.35	12.35

[1] Calculated from formula $y = 42.62(1 - 0.0692)^x$.
[2] Calculated from the formula $y = 54.9(1 - 0.0579)^x$.

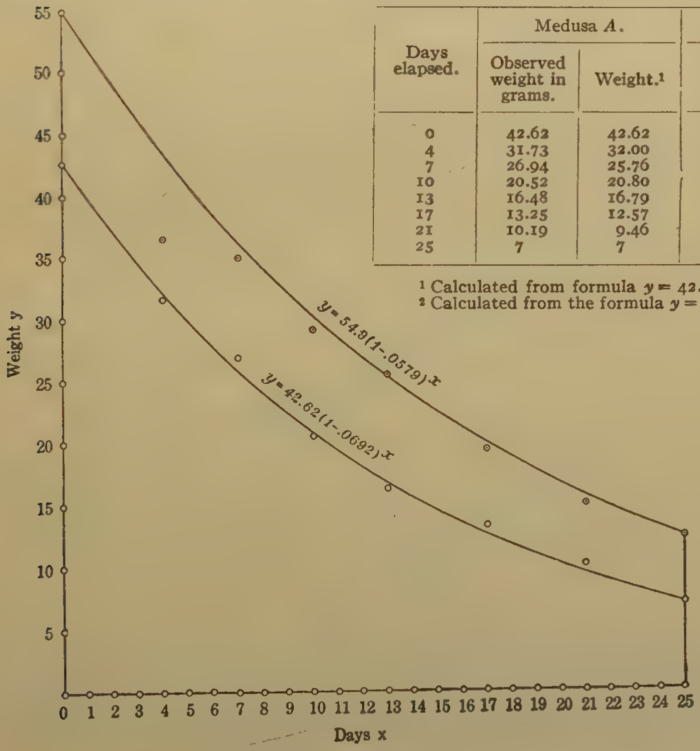

Diagram illustrating Table 9.

Table 10 shows the decline in weight of 6 normal medusæ of *Cassiopea xamachana* starved 26 days in filtered sea-water in the *dark* at temperatures ranging from 27.3° to 30.1° C., from June 18 to July 14, 1911, at Tortugas, Florida.[1] The medusæ were placed in a 6-liter glass aquarium, the water being changed once in each 24 hours. The temperature conditions were similar to those maintained for the medusæ whose rates of decline are shown in table 4.

TABLE 10.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	85.59	85.6	9	41.03	42.47	18	23.24	20.97
1	79.57	79.18	10	40.96	39.20	19	20.965	19.43
2	75	73.27	11	39.4	36.29	20	18.85	17.98
3	70.9	67.71	12	33.535	33.56	21	17.56	16.69
4	63.35	62.66	13	28.51	31.07	22	15.31	15.41
5	56.2	57.87	14	27.23	28.76	23	14.38	14.21
6	52.6	53.59	15	27.12	26.54	24	13.07	13.18
7	51.705	49.56	16	26.49	24.57	25	10.78	12.16
8	42.09	45.88	17	24.87	22.77	26	8.99	11.30

[1] According to the formula $y = 85.6(1 - 0.075)^x$.

REMARKS.—These 6 medusæ were captured in the moat of Fort Jefferson, Tortugas, on June 16 and weighed for the first time on June 18, 1911. The smallest was about 25 mm. wide on June 18. On June 22 the smallest was only 8.5 mm. wide, and by June 25 and 26 the medusæ had begun to macerate.

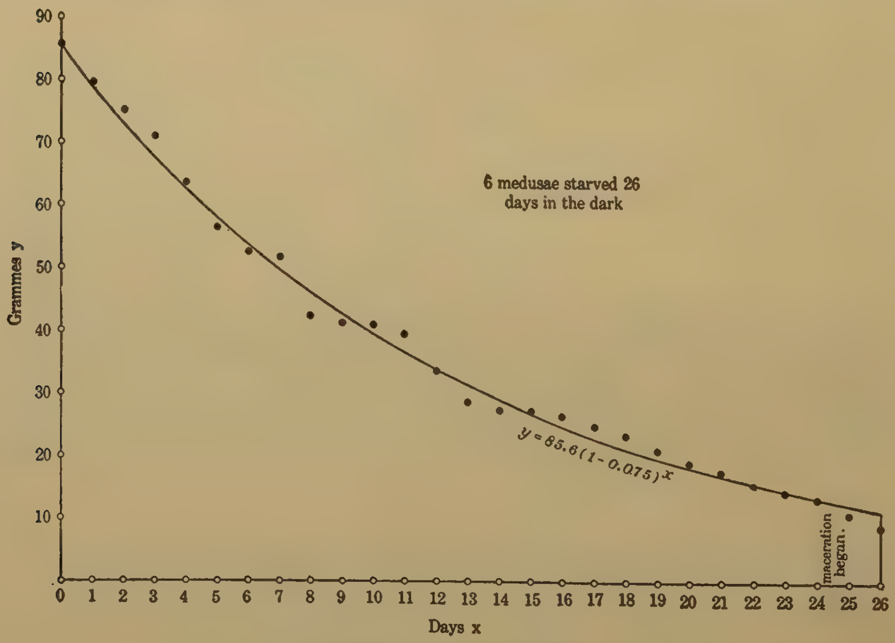

Diagram illustrating Table 10.

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[1] For the first 14 days the medusæ remained in complete darkness, not being exposed for an instant to the light. On the fourteenth day they were observed in diffuse light, and it appeared that they were all blue-translucent, not greenish gray, as are the normal medusæ. The algal cells were then greatly reduced in number. The medusæ were then replaced in the dark as soon as possible, and remained in darkness until the end of the experiment, excepting that from the fourteenth to the twenty-sixth day they were weighed in the diffuse light of the laboratory but were replaced in the dark room immediately after each weighing.

Table 11 shows the decline in weight of a single medusa of *Cassiopea xamachana* starved 19 days in filtered sea-water, in the *dark*, at temperatures ranging between 27.3° and 30.1° C., from June 30 to July 19, 1911, at Tortugas, Florida. During the first 10 days this medusa was placed in 400 c.c. of sea-water which was changed once each day. After this it was placed in a glass vessel holding about 6 liters and the water was changed constantly by mechanical means, thus giving an abundant supply of fresh sea-water. Compare this with table 12 which shows the starving rate of a medusa maintained in daylight.

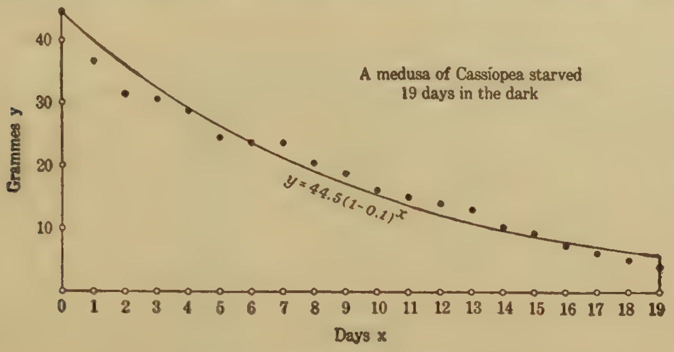


Diagram illustrating Table 11.

TABLE 11.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]	Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	44.51	44.5	7	23.66	21.27	14	10.45	10.19
1	36.69	40.05	8	20.49	19.13	15	9.45	9.17
2	31.44	36.24	9	18.99	17.24	16	7.70	8.25
3	30.59	32.44	10	16.05	15.53	17	6.30	7.39
4	28.825	29.19	11	15.03	13.97	18	5.23	6.68
5	24.485	26.25	12	14.01	12.57	19	4.23	6
6	23.77	23.63	13	13.12	11.303	....	....	....

[1] According to the formula $y = 44.5(1 - 0.1)^x$.
REMARKS.—On July 19 maceration began and the experiment was discontinued.

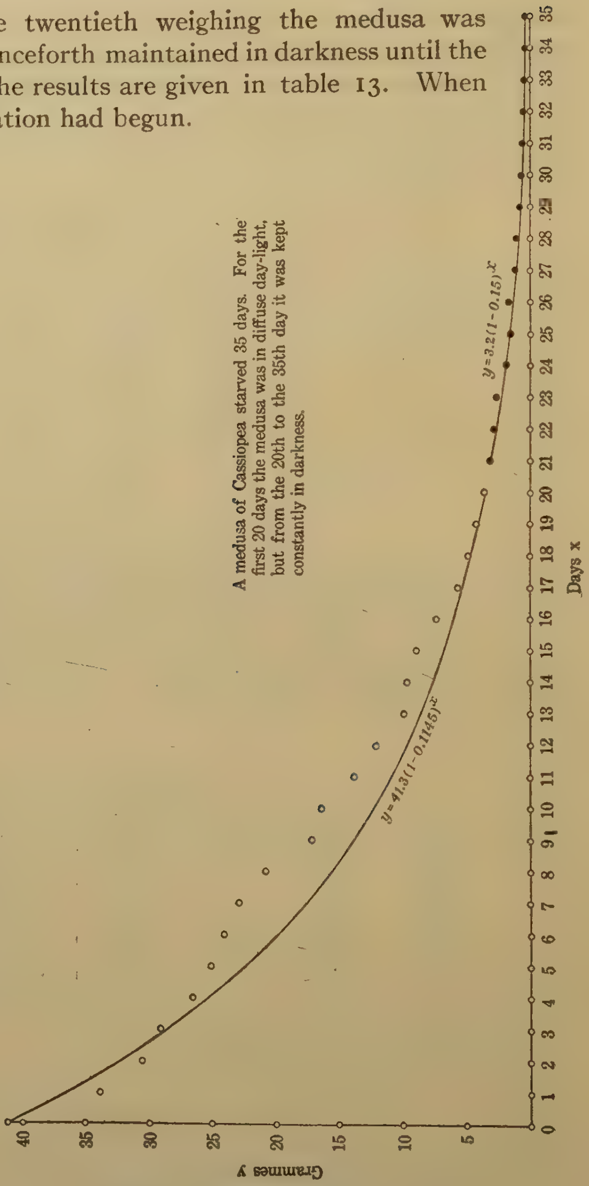
Table 12 shows the decline in weight of a single medusa of *Cassiopea xamachana* starved 20 days in the *diffuse daylight* of the laboratory, and then for 15 more days in the dark, in filtered sea-water at temperatures ranging between 27.3° and 30.1° C., from June 30 to August 4, 1911, at Tortugas, Florida. During the first 10 days this medusa was placed in 400 c.c. of sea-water which was changed once each day. After this it was placed in a glass vessel holding about 6 liters and the water was changed constantly by mechanical means, thus giving an abundant supply of sea-water. The conditions of the experiment are comparable with those shown on table 11, excepting that table 12 applies to a medusa starved in daylight, while table 11 shows the decline in weight of one starved in darkness.

Immediately after the twentieth weighing the medusa was placed in the dark and thenceforth maintained in darkness until the end of the experiment. The results are given in table 13. When the records ceased, maceration had begun.

TABLE 12.

Days elapsed.	Observed weight in grams.	Calculated weight.[1]
0	41.32	41.3
1	33.92	36.55
2	30.52	32.38
3	29.18	28.66
4	26.66	25.4
5	25.09	22.47
6	24.03	19.91
7	22.93	17.64
8	20.77	15.61
9	17.63	13.84
10	16.41	12.22
11	13.80	10.82
12	12.02	9.58
13	9.86	8.51
14	9.66	7.52
15	8.93	6.65
16	7.34	5.91
17	5.64	5.20
18	4.875	4.63
19	4.27	4.09
20	3.66	3.63

[1] According to formula $y = 41.3(1 - 0.1145)^x$.



A medusa of Cassiopea starved 35 days. For the first 20 days the medusa was in diffuse day-light, but from the 20th to the 35th day it was kept constantly in darkness.

Diagram illustrating Tables 12 and 13.

TABLE 13.

Days elapsed in darkness.		Observed weight.	Calculated weight.[1]	Days elapsed in darkness.		Observed weight.	Calculated weight.[1]	Days elapsed in darkness.		Observed weight.	Calculated weight.[1]
21	1	3.2	3.2	26	6	1.54[2]	1.42	31	11	0.68	0.64
22	2	2.83	2.72	27	7	1.205	1.206	32	12	0.54	0.54
23	3	2.53	2.31	28	8	1.01	1.02	33	13	0.54	0.45
24	4	1.98	1.96	29	9	0.87	0.84	34	14	0.45	0.39
25	5	1.52	1.64	30	10	0.75	0.74	35	15	0.29	0.34

[1] According to the formula $y = 3.2(1 - 0.15)^x$.

[2] An error in weighing.

Table 14 shows the decline in weight of a normal medusa of *Cassiopea xamachana* starved 25 days in the *dark*, in filtered sea-water, at 27.5° to 30° C., from June 28 to July 23, 1912, at Tortugas, Florida. The medusa was placed in a glass aquarium holding 6 liters, the water being changed once in each 24 hours.

TABLE 14.

Days elapsed.	Observed weight of medusa in grams.	Calculated. weight.[1]	Days elapsed.	Observed weight of medusa in grams.	Calculated weight.[1]
0	32.77	32.77	13	15.09	13.93
4	21.58	25.17	17	12.52	10.65
7	19.54	20.61	21	9.04	8.12
10	17.34	16.91	25	6.28	6.28

[1] According to formula $y = 32.77(1 - 0.0639)^x$.

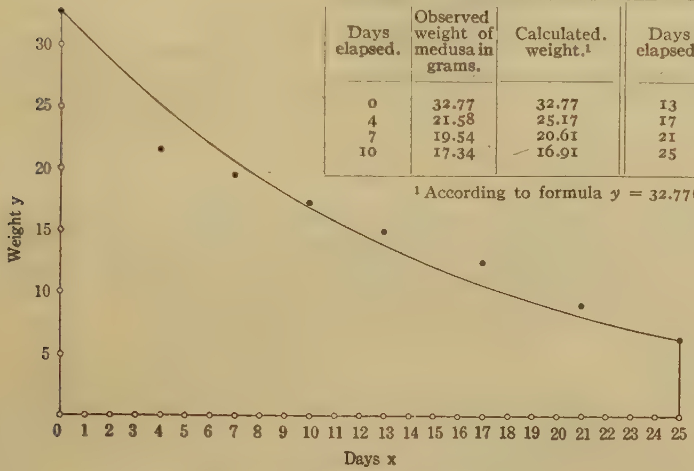


Diagram illustrating Table 14.

Table 15 shows the decline in weight of two medusæ of *Cassiopea xamachana* starved in *diffuse daylight* in constantly changing, filtered sea-water at temperatures ranging from 27.3° to 30.1° C., at Tortugas, Florida, from July 10 to August 4, 1911. Compare this with table 16.

These medusæ were starved side by side in the same 6-liter glass vessel and were subjected to the same changes of temperature, water, light, etc. Being in running water, they lost weight more rapidly than one would expect had they been in semi-stagnant water.

TABLE 15.

Days elapsed.	Medusa *A*.		Medusa *B*.		Days elapsed.	Medusa *A*.		Medusa *B*.	
	Observed weight.	Expected weight.[1]	Observed weight.	Expected weight.[2]		Observed weight.	Expected weight.[1]	Observed weight.	Expected weight.[2]
0	40.58	40.6	37.16	37.1	13	10.26	10.11	8.20	9.18
1	40.26	36.48	33.5	33.37	14	9.34	9.05	7.67	8.18
2	37.08	32.76	26.88	29.95	15	8.56	8.16	7.38	7.39
3	31.6	29.43	23.53	26.90	16	8.09	7.31	7.00	6.65
4	27.03	26.47	20.03	24.15	17	7.21	6.58	6.515	5.95
5	23.475	23.79	18.35	21.70	18	6.09	5.93	5.39	5.35
6	19.79	21.36	16.53	19.47	19	5.41	5.32	4.73	4.79
7	18.15	19.20	13.78	17.50	20	4.73	4.75	3.99	4.31
8	17.41	17.25	13.48	15.72	21	4.17	4.26	....	....
9	14.17	15.47	12.43	14.12	22	3.99	3.85	....	....
10	12.79	13 93	11.19	12.67	23	3.48	3.46	....	....
11	11.92	12.50	10.30	11.37	24	3.08	3.11	....	....
12	10.96	11.25	8.80	10.22	25	2.52	2.79	....	....

[1] Calculated from formula $y = 40.6(1 - 0.1015)^x$.
[2] Calculated from formula $y = 37.16(1 - 0.102)^x$.

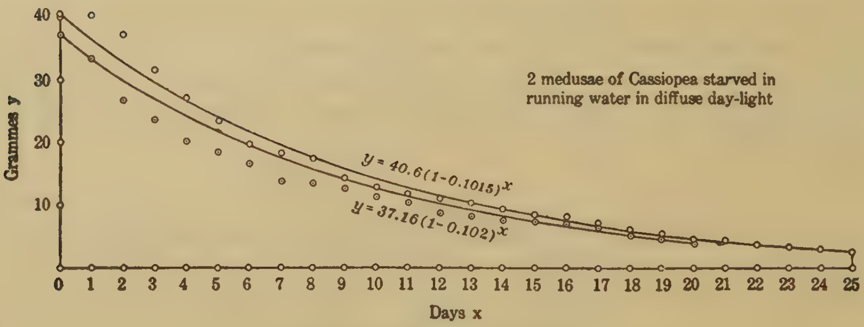


Diagram illustrating Table 15.

Table 16 shows the decline in weight of two medusæ of *Cassiopea xamachana* starved 9 days in the *dark* in constantly changing, filtered sea-water at temperatures ranging from 27.3° to 30.1° C., at Tortugas, Florida, from July 10 to 19, 1911. Compare this with table 15.

TABLE 16.

Days elapsed.	Medusa *C*.		Medusa *D*.	
	Observed weight.	Expected weight.[1]	Observed weight.	Expected weight.[2]
0	51	....	36.37	36.37
1	[3]51.78	51	34.17	29.64
2	44.09	42.58	24.88	24.15
3	38.86	35.55	20.83	19.68
4	30.5	29.68	16.88	16.04
5	28.28	24.79	15.33	13.06
6	24.13	20.71	14.19	10.66
7	19.25	17.29	9.62	8.69
8	17.61	14.43	7.49	7.06
9	11.97	12.03	5.79	5.78

[1] According to formula $y = 51(1 - 0.165)^x$.

[2] According to formula $y = 36.37(1 - 0.185)^x$.

[3] Medusa *C* did not begin to lose weight until after it had been confined 24 hours in the dark. The commensal plant-cells seem to be of no aid to the medusa when in pure, constantly changing sea-water. The medusæ live, in nature, only in semi-stagnant salt lagoons.

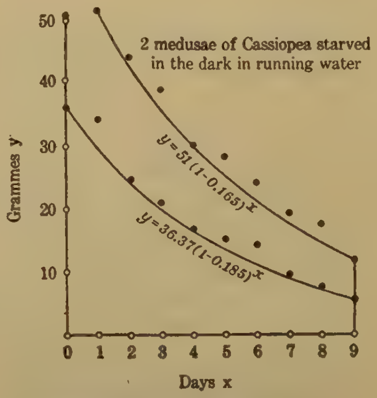


Diagram illustrating Table 16.

TABLE 17.

Days elapsed.	Medusa *A*.		Medusa *B*.		Medusa *C*.	
	Weights in water which had been tested to 70° C.	Calculated weights.[1]	Weights in water which had been partially exhausted of air.	Calculated weights.[2]	Weights in normal filtered sea-water.	Calculated weights.[3]
0	49.567	50	40.45	....	35.83	35.8
2	46.61	44.62	40.69	40.5	29.94	31.82
4	45.13	39.9	[4]38.57	36.08	28.44	28.32
6	38.39	35.6	32.18	32.19	24.61	25.16
8	33.09	31.8	31.64	28.7	22.94	22.37
10	29.7	28.3	29.28	25.55	20.93	19.95
12	26.19	25.25	25.73	22.76	19.18	17.61
14	23.7	22.7	21.82	20.29	16.77	15.65
16	19.83	20.2	18.28	18.1	13.93	13.99
18	18.18	17.8	16.51	16.51	12.45	12.45
20	15.7	15.4	14.36	14.75	11.1	11.06

[1] According to the formula $y = 50(1 - 0.0553)^x$.

[2] According to the formula $y = 40.5(1 - 0.0559)^x$.

[3] According to the formula $y = 35.8(1 - 0.057)^x$.

[4] Probably an error.

Table 17 shows the decline in weight of three normal specimens of *Cassiopea xamachana* starved for 20 days at Tortugas, Florida, in filtered sea-water. One medusa (*A*) was maintained in water which had been heated to 71° C. and then cooled to normal temperatures. Another (*B*) was kept in sea-water from which most of the dissolved air had been exhausted by means of an air pump. The third medusa (*C*) was kept in normal filtered sea-water.

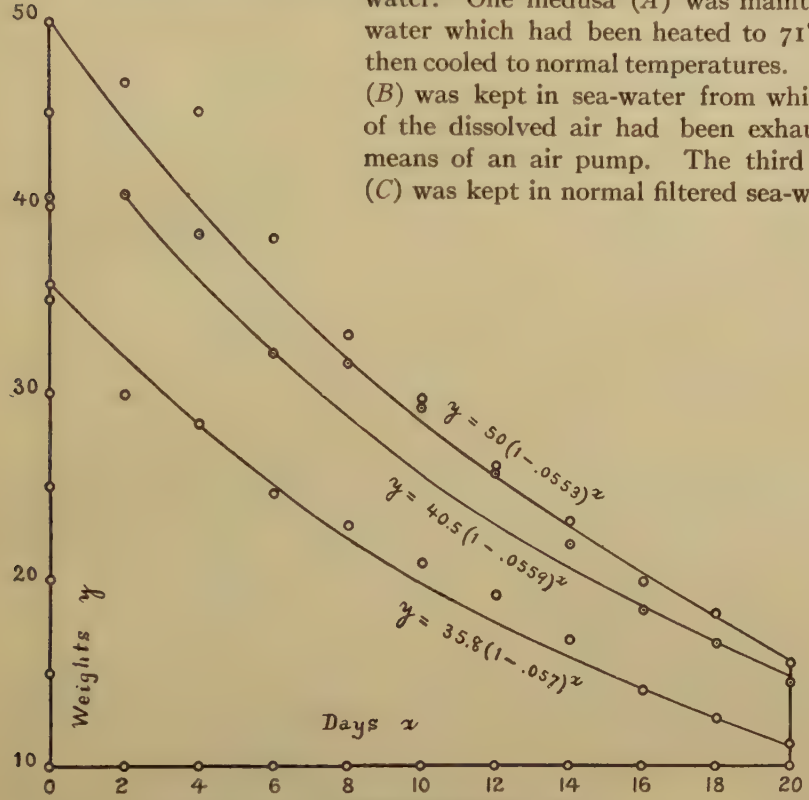


Diagram illustrating Table 17.

Table 18 shows the decline in weight of three half disks of *Cassiopea xamachana* each starved in 6 liters of water in diffuse daylight, together with

TABLE 18.

Days elapsed.	Disk *A*.		Disk *B*.		Disk *C*.	
	Weights of half-disk starved in sea-water which had been heated to 71° C.	Weights calculated.[1]	Weights of half-disk starved in sea-water which had been partially exhausted of air.	Weight calculated.[2]	Half of disk starved in normal filtered sea-water.	Weight calculated.[3]
0	23.05	23.05	20.035	20.03	18.535	18.5
2	19.46	20.33	17.07	17.68	15.68	16.27
4	17.815	17.96	14.72	15.62	13.83	14.27
6	15.55	15.89	12.33	13.82	11.84	12.52
8	13.82	14	11.38	12.2	11.04	11
10	12.16	12.4	10.17	10.8	9.04	9.65
12	10.63	11.1	9.31	9.66	7.7	8.57
14	9.68	9.69	8.39	8.42	7.21	7.43
16	8.79	8.55	7.63	7.44	6.4	6.52
18	7.61	7.56	6.84	6.58	5.65	5.72
20	6.71	6.71	6.2	5.82	5.03	5.03

[1] According to formula $y = 23(1 - 0.06)^x$.
[2] According to formula $y = 20(1 - 0.06)^x$.
[3] According to formula $y = 18.5(1 - 0.063)^x$.

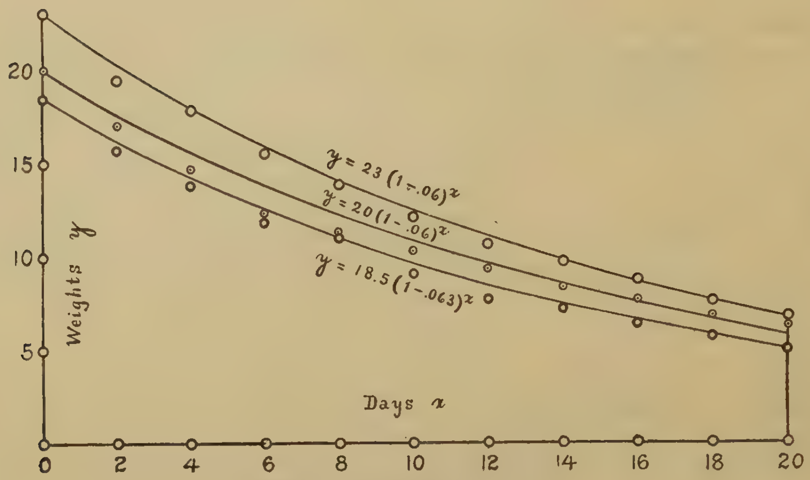


Diagram illustrating Table 18.

the normal medusæ described on table 17. The stomach and mouth-arms were cut off and the disk cut into halves. One half (Disk *A*) was starved in doubly filtered sea-water which had been heated to 71° C.; the other half of the same disk (Disk *B*) was starved in doubly filtered sea-water which had been placed in a vacuum to expel most of its dissolved air. The third half disk (Disk *C*) was taken from another medusa and was starved in normal doubly filtered sea-water.

Table 19 shows the decline in weight of two regenerating specimens of *Cassiopea xamachana* starved 19 days in diffuse daylight, in filtered sea-water

TABLE 19.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of disk.[1]	Weight of regenerating medusa in grams.	Weight of regenerating normal medusa.[2]
0	25.48	25.48	41.87	41.87
2	20.15	20.48	33.98	36.2
6	9.66	13.24	24.30	27.3
9	8	9.55	18.97	22.02
12	6.52	6.88	14.23	17.79
15	5.06	4.97	13.87	14.36
19	4.21	3.21	10.81	10.81

[1] Calculated according to formula $y = 25.48(1 - 0.1034)^x$.
[2] Calculated according to formula $y = 41.87(1 - 0.0689)^x$.

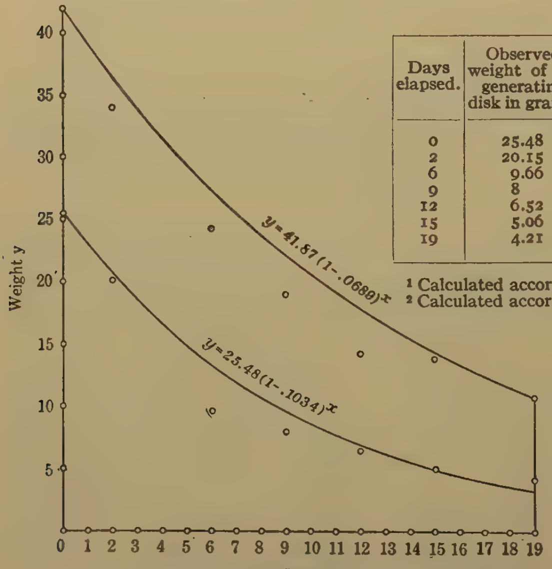


Diagram illustrating Table 19.

at 27.5° to 30° C., from June 26 to July 15, 1912, at Tortugas, Florida. One of these specimens was a disk from which the stomach and mouth-arms had been cut off, and the other was a medusa with stomach and mouth-arms intact but with its bell-rim cut off. The two were maintained in one and the same 6-liter glass aquarium jar throughout the experiment, and were thus subjected to identical environmental conditions. The water was changed once in each 24 hours.

Table 20 shows the decline in weight of two regenerating species of *Cassiopea xamachana* starved 19 days in *diffuse daylight,* in filtered sea-water at 27.5° to 30° C., from June 26 to July 15, 1912, at Tortugas, Florida. One of these specimens was a disk from which the stomach and mouth-arms had been removed, and the other specimen was a medusa with stomach and mouth-arms intact but with the bell-rim removed. The two were maintained in one and the same 6-liter glass aquarium throughout the experiment, and were thus subjected to identical environmental conditions. The water was changed once in every 24 hours.

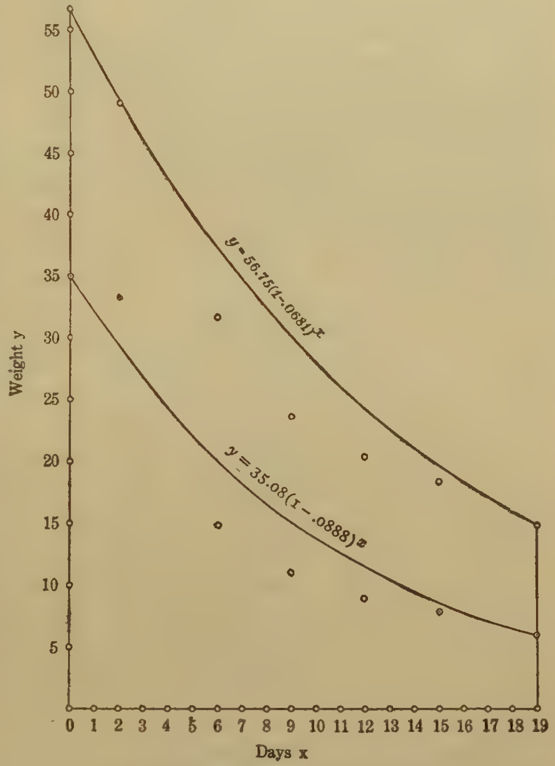


Diagram illustrating Table 20.

TABLE 20.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of disk.[1]	Observed weight of regenerating medusa in grams.	Weight of regenerating medusa.[2]
0	35.08	35.08	56.75	56.75
2	33.28	29.11	49.11	49.26
6	14.86	20.06	31.76	37.17
9	11.04	15.19	23.63	30.07
12	8.98	11.51	20.43	24.34
15	7.9	8.7	18.45	19.69
19	6	6	14.88	14.88

[1] Calculated according to formula $y = 35.08(1 - 0.0888)^x$.
[2] Calculated according to formula $y = 56.75(1 - 0.0681)^x$.

Table 21 shows the decline in weight of the two halves of a disk of *Cassiopea xamachana* starved in the diffuse daylight of the laboratory at Montego Bay, Jamaica, from February 27 to March 18, 1912, in water ranging from 24.5° to 28° C. The stomach and mouth-arms of the medusa were removed and the disk cut into two nearly equal halves, both of which were maintained in one and the same glass aquarium holding about 6 liters. The water was changed once in every 24 hours.

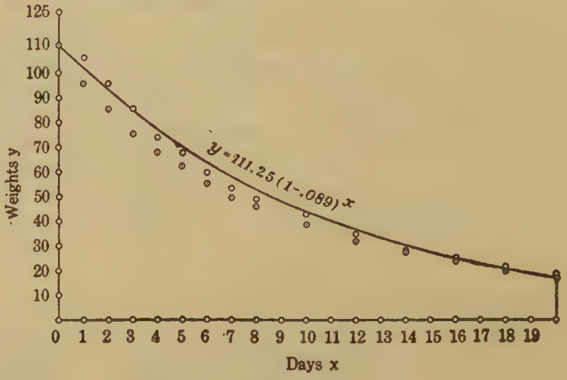


Diagram illustrating Table 21.

TABLE 21.

Days elapsed.	Medusa *A*.		Medusa *B*.	
	Weight.	Calculated weight.[1]	Weight.	Calculated weight.[2]
0	111.25	111.25	125.93	125.93
1	95.61	101.35	106.13	114.33
2	85.41	92.34	95.61	103.76
3	75.43	84.11	85.33	94.32
4	67.96	76.66	73.96	85.62
5	62.39	69.77	67.51	77.69
6	55.29	63.63	59.81	70.52
7	49.53	57.96	53.06	64.09
8	45.76	52.73	48.86	58.18
10	38.49	43.83	42.72	47.97
12	32.84	36.38	34.56	39.53
14	27.31	30.15	28.65	32.61
16	23.88	25.03	25.08	26.81
18	19.76	20.8	21.61	22.16
20	17.12	17.24	18.26	18.27

[1] According to the formula $y = 111.25(1 - 0.089)^x$.
[2] According to the formula $y = 125.93(1 - 0.092)^x$.

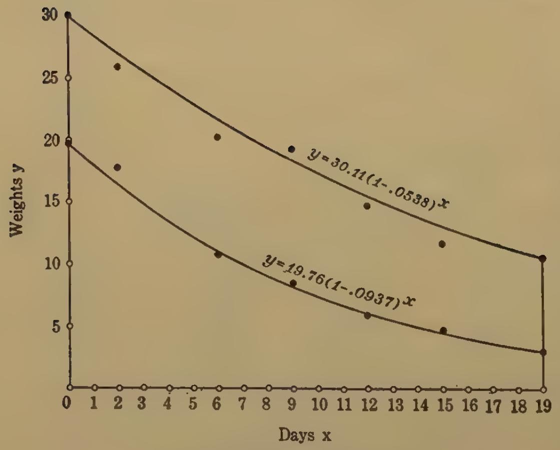


Diagram illustrating Table 22.

Table 22 shows decline in weight of two regenerating specimens of *Cassiopea xamachana* starved 19 days in the *dark*, in filtered sea-water, at temperatures ranging from 27.5° to 30°C., from June 26 to July 15, 1912, at Tortugas, Florida. One was a disk deprived of its stomach and mouth-arms, and the other was a medusa regenerating its bell-rim, but with stomach and mouth-arms intact.

TABLE 22.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of disk.[1]	Observed weight of regenerating medusa in grams.	Weight of regenerating medusa.[2]
0	19.76	19.76	30.11	30.11
2	17.8	16.22	25.89	26.95
6	10.8	10.95	20.21	21.59
9	8.54	8.14	19.22	18.31
12	5.94	6.07	14.76	15.5
15	4.79	4.62	11.7	13.13
19	3.05	3.05	10.53	10.53

[1] Calculated according to formula $y = 19.76(1 - 0.0937)^x$.
[2] Calculated according to formula $y = 30.11(1 - 0.0538)^x$.

The two specimens were maintained in one and the same 6-liter aquarium jar throughout the experiment, and were thus subjected to identical environmental conditions. The water was changed once in every 24 hours.

Table 23 shows the decline in weight of two regenerating specimens of *Cassiopea xamachana*, one being a disk without stomach or mouth-arms, and the other a medusa with stomach and mouth-arms intact but with the rim of the bell cut off. Thus each medusa was regenerating, the disk being without and the medusa with its stomach. These two specimens were kept side by side in one and the same 6-liter glass aquarium, in *constant darkness*, in filtered sea-water at 27.5° to 30° C. for 19 days from June 26 to July 15, 1912. The water was changed once in every 24 hours.

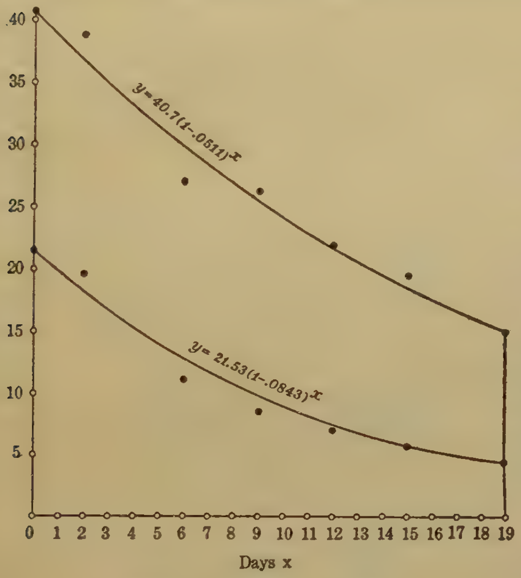


Diagram illustrating Table 23.

TABLE 23.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of regenerating disk.[1]	Observed weight of regenerating medusa in grams.	Weight of regenerating medusa.[2]
0	21.53	21.53	40.7	40.7
2	19.59	18.02	38.8	36.65
6	11.12	12.83	27.12	29.72
9	8.56	9.74	26.33	25.4
12	7.07	7.46	21.96	21.71
15	5.76	5.74	19.48	18.55
19	4.04	4.04	15.05	15.05

[1] Calculated according to formula $y = 21.53(1 - 0.0843)^x$.
[2] Calculated according to formula $y = 40.7(1 - 0.0511)^x$.

Table 24 shows the decline in weight of two regenerating specimens of *Cassiopea xamachana* starved 19 days in the *dark*, in filtered sea-water, at temperatures ranging from 27.5° to 30° C., from June 26 to July 15, 1912, at Tortugas, Florida. One of the specimens was a disk deprived of both stomach and mouth-arms, and the other was a medusa with the rim of its bell cut off but with stomach and mouth-arms intact; thus both were regenerating new tissue. They were kept side by side in one and the same 6-liter aquarium jar, and were thus subjected to similar conditions. The water was changed once in every 24 hours.

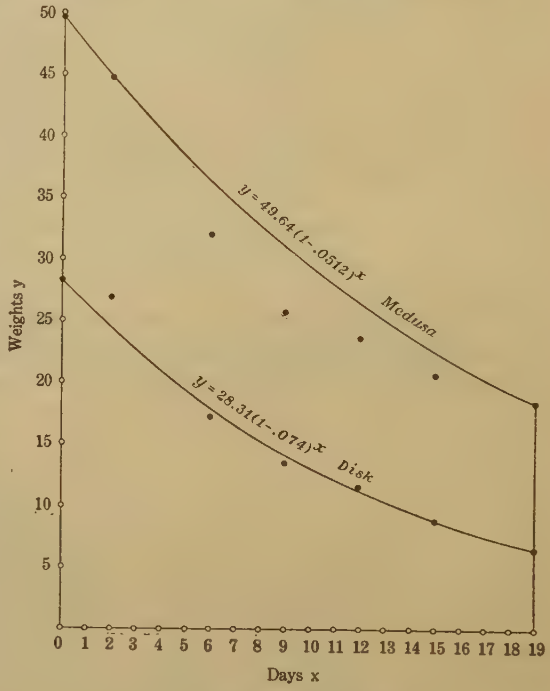


Diagram illustrating Table 24.

TABLE 24.

Days elapsed.	Observed weight of regenerating disk in grams.	Weight of disk.[1]	Observed weight of regenerating medusa in grams.	Weight of regenerating medusa.[2]
0	28.31	28.31	49.64	49.64
2	26.91	24.25	44.61	44.65
6	17.23	17.83	31.97	36.19
9	13.57	14.16	25.71	30.88
12	11.64	11.24	23.61	26.36
15	8.8	8.91	20.62	22.51
19	6.58	6.58	18.24	18.24

[1] Calculated according to formula $y = 28.31 (1 - 0.074)^x$.
[2] Calculated according to formula $y = 49.64(1 - 0.0512)^x$.

Table 25 shows the decline in weight of the two halves of a disk of *Cassiopea xamachana* without stomach or mouth-arms. One of these halves, *A*, was starved in the diffuse daylight of the laboratory and the other, *B*, in constant darkness, for 18 days, from February 28 to March 18, 1912, at Montego Bay, Jamaica, at temperatures ranging from 24.5° to 28° C. The two halves were maintained side by side in one and the same 6-liter glass aquarium and the water was changed once in each 24 hours.

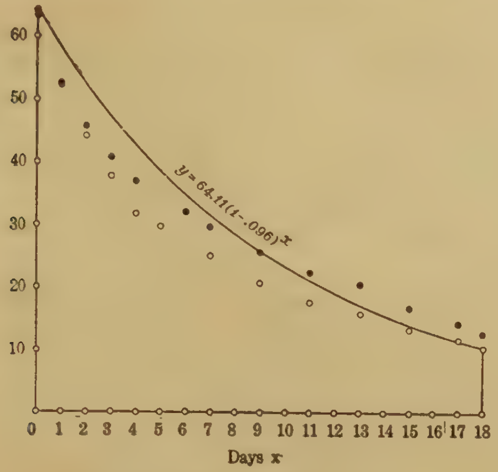


Diagram illustrating Table 25.

TABLE 25.

Days elapsed.	Medusa *A*.		Medusa *B*.	
	Weight, starved in diffuse daylight.	Weight.[1]	Weight, starved in darkness.	Weight.[2]
0	64.11	64.11	63.46	63.46
1	52.18	57.96	52.53	58.16
2	44.28	52.38	45.91	53.12
3	37.88	47.38	40.93	48.61
4	31.98	42.83	37.06	44.48
5	29.9	38.72	....	....
6	....	....	32.27	37.25
7	25.07	31.61	29.91	34.08
9	20.9	25.83	25.69	28.56
11	17.69	21.09	22.49	23.35
13	15.83	17.24	20.6	20.
15	13.45	14.11	16.73	16.75
17	11.86	11.54	15.46	14.02
18	10.40	10.45	12.83	12.83

[1] According to formula $y = 64.11(1 - 0.096)^x$.
[2] According to formula $y = 63.46(1 - 0.085)^x$.

TABLE 26.

Days elapsed.	Half-disk starved in daylight.	Decline in weight.[1]	Half-disk starved in dark.	Decline in weight.[2]
0	73.01	73	66.11	66.11
1	63.13	65.12	58.28	58.83
2	53.06	58.11	51.33	52.35
3	41.49	51.83	43.09	46.60
4	33.91	46.21	37.11	41.44
5	31.01	41.25	....	36.88
6	....	....	30	32.85
7	26.51	32.78	25.66	29.22
8	....	....	....	....
9	22.44	26.06	19.12	23.14
10	....	....	....	....
11	18.56	20.8	16.87	18.31
12	....	....	....	....
13	16.22	16.50	14.83	14.54
14	....	....	....	....
15	13.47	13.14	12.06	11.5
16	....	....	....	....
17	11.17	10.44	10.54	9.12
18	9.40	9.34	8.06	8.06

[1] Calculated in accordance with formula $y = 73(1 - 0.108)^x$.
[2] Calculated according to formula $y = 66.11(1 - 0.11)^x$.

Table 26 shows the decline in weight of two halves of a disk of *Cassiopea frondosa* deprived of its stomach. One of these halves was starved in diffuse daylight and the other in the dark from February 28 to March 18, 1912, at Montego Bay, Jamaica.

TABLE 27.

Days elapsed.	Medusa *A*.		Medusa *B*.	
	Observed weights.	Weights.[1]	Observed weights.	Weights.[2]
0	60.96	61	57.54	57.5
2	51.17	50.08	49.3	49.45
4	42.26	42.27	45.36	42.26
6	34.01	35.19	39.06	36.51
8	29.33	29.33	31.41	31.41
10	23.59	24.77	27.6	27.25
12	21.2	20.37	24.72	23.23

[1] Calculated from the formula $y = 61(1-0.1673)^x$.
[2] Calculated from the formula $y = 57.5(1-0.14)^x$.

Table 27 shows the decline in weight of two normal medusæ of *Cassiopea xamachana* starved each in one liter of sea-water, changed once in each 24 hours, and kept in the diffuse daylight of the laboratory at Tortugas, Florida, from June 8 to 20, 1913. One medusa, *A*, was starved in sea-water which had been passed through two glass funnels each holding two sheets of Chardin filter paper. The other medusa, *B*, was starved in sea-water, which, in addition to having been filtered through the aforesaid Chardin filters, was also filtered through a bacteria-proof porcelain filter. It appears that all food had been removed from the water by the Chardin filters and the medusa in the bacteria-free sea-water starved somewhat more slowly than the one in the sea-water which had not been passed through the porcelain filter.

DESCRIPTION OF PLATE.

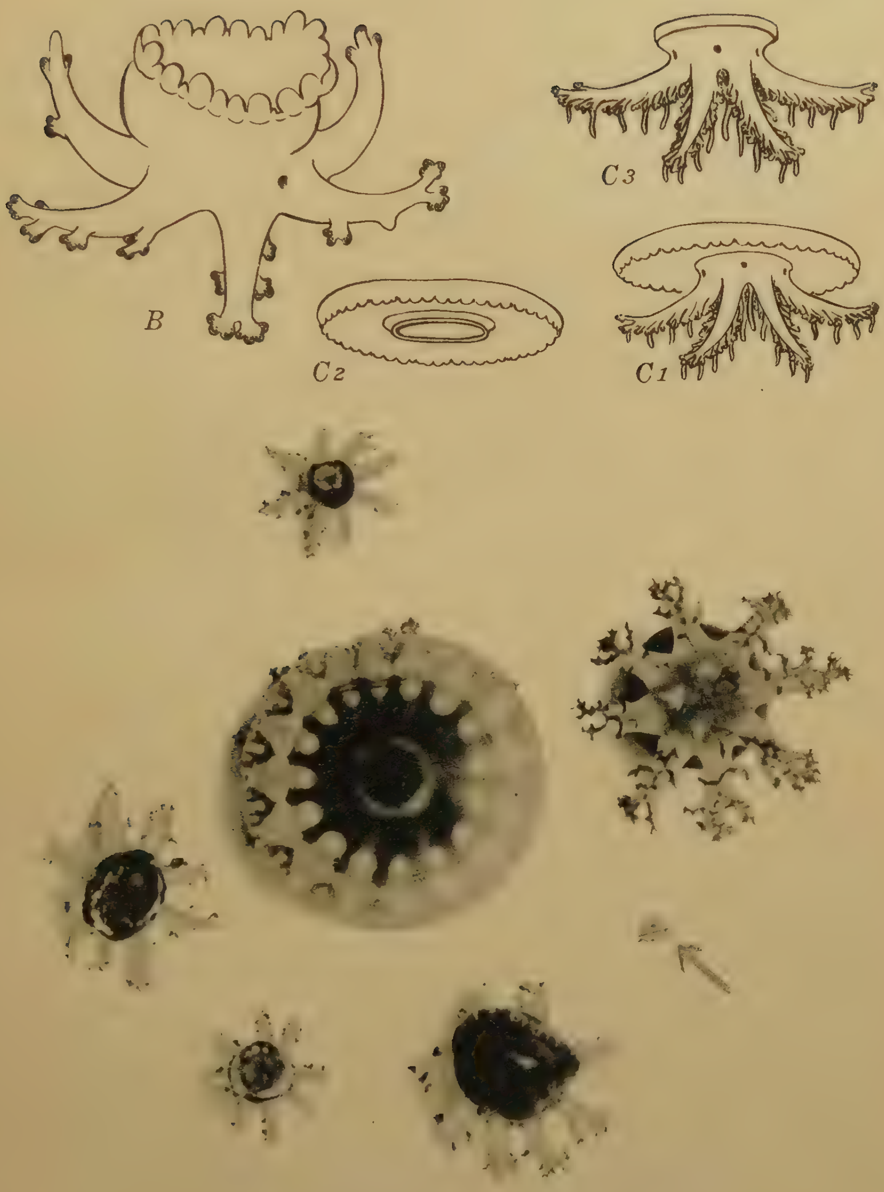
FIG. A. *Cassiopea xamachana.* The 6 medusæ have been starved for 41 days in diffuse daylight (see table 4). The recently caught, unstarved medusa in the center of the aquarium is of nearly the same size that the smallest (shown by the arrow) was when starving began. The dark-brown color of the starving medusæ is due to their densely crowded commensal plant-cells.

FIG. B. *Cassiopea xamachana* which has been starved for 41 days in darkness. Originally its bell was 25 mm. wide; now it is only 4.5 mm. in diameter, and the rim is turned upward and inward. The mouth-arms are each 3 mm. long, and the mouths have disappeared by coalescence of the lips. The medusa is still pulsating, a single rhopalium functioning.

FIG. C_1. Normal medusa of *Cassiopea xamachana.*

FIG. C_2. Disk without stomach or mouth-arms.

FIG. C_3. The stomach and mouth-arms of the medusa, the disk having been cut away.


B
C3
C2
C1
A

IV.

CHANGES IN SALINITY AND THEIR EFFECTS UPON THE REGENERATION OF CASSIOPEA XAMACHANA.

BY A. J. GOLDFARB,
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Four figures.

CHANGES IN SALINITY AND THEIR EFFECTS UPON THE REGENERATION OF CASSIOPEA XAMACHANA.

By A. J. Goldfarb.

Cassiopea xamachana, a large scyphomedusa, is very abundant in the very shallow waters of the moat at Fort Jefferson, Dry Tortugas, Florida. The conditions in this moat are such that the medusæ are in all probability subjected to extreme changes in salinity, due on the one hand to evaporation in the subtropical sun, to the shallow water and inclosed nature of the moat, to the single tide, etc., and on the other hand to extreme dilution due to the subtropical rains. Doctors A. G. Mayer and T. W. Vaughan have undertaken a study of these changes in salinity in and outside of the moat, under various conditions of tide and season. Until their report is forthcoming, this phase of the problem must be left in abeyance.

The present report considers to what extent changes in salinity influence regeneration in *Cassiopea*, and the results of the investigation are compared with those previously obtained with the hydroid *Eudendrium ramosum* of Woods Hole, Massachusetts, and with the observations of Loeb with the hydroid *Tubularia* of Serino Bay, Italy.

Though certain variable factors are known to accelerate, retard, or modify the regeneration of an organism, these factors have not heretofore been sufficiently taken into account. In the present investigation much care was taken to exclude or make the following factors uniform:

1. *Size of medusæ.* Size roughly indicates age, and as the rate of growth varies with age the medusæ finally chosen were approximately of the same size. Each solution contained one of medium size, 100 to 105 mm. in diameter, and one smaller medusa, 85 to 90 mm. in diameter.

2. *Volume, surface, and depth of solution.* To insure uniformity of respiration and evaporation, and to prevent excessive concentration, the medusæ were kept in jars of equal size and shape, each jar containing 2,000 c.c. of the solution.

3. *Aeration and change of solution.* All solutions were shaken thoroughly to aerate them, and were changed daily to avoid excess CO_2, excess evaporation, and bacteria.

4. *Degree of injury.* It has been repeatedly urged that the rate of regeneration is influenced by the extent of injury. Though recent evidence is opposed to this view, its possibility was taken into account by removing the same number of arms from each pair of medusæ.

5. *Level of amputation* has repeatedly been shown to affect considerably the rate and nature of the regenerated organ. In these experiments all arms were amputated at the same level, namely, at their respective bases.

6. *Temperature.* By avoiding local heat and local drafts the temperature was made uniform for the series.

7. *Preparation of the solutions.* To avoid disturbing the "protective relation" of the salts and to avoid introducing any salt not normally present in sea-water, the solutions were made as follows: The dilute solutions were made by adding enough (rain) water to make solutions containing 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, and 40 per cent of sea-water. The rain-water used in this series was tested by Dr. A. G. Mayer, who, upon the basis of the chlorine content, calculated that it contained 0.001 part of sea-water, an amount entirely negligible, as shown by these and other experiments. The concentrated series was made by slow evaporation in the sun's heat. In this manner, solutions containing 5, 11, 17, 25, 33, 42, and 53 per cent more than the normal salts in sea-water were obtained.

8. *The number of arms amputated* totaled 323.

9. *Three sets of measurements were recorded,* 14, 24, and 30 days after amputation. These measurements are given in table 1.

TABLE 1.—*The influence of changes in salinity on regeneration.*

Per cent of sea-water in solution.	No. of amputated arms.	Average regeneration in mm.			Remarks.
		14 days.	24 days.	30 days.	
153	15	0	0	0	
142	15	0	0	0.12	Regeneration abnormal.
133	15	0	0	0	
125	14	0.16	0	0.66	
117	15	0.21	0.62	0.43	
111	15	4.32	4.23	1.92	
105	14	3.72	3.00	2.19	Regeneration normal.
100	43	3.41	6.44	4.82	
95	15	5.46	8.41	5.69	
90	15	3.60	7.22	5.00	
85	15	4.60	7.17	5.05	
80	13	3.02	5.33	3.45	
75	15	1.87	6.09	3.74	
70	15	2.22	5.41	3.63	Regeneration abnormal.
65	15	1.26	3.64	3.33	
60	14	0.87	2.06	2.20	
55	15	0.44	1.57	2.87	
50	15	0.50	0	0	
45	15	0	0	0	
40	15	0	0	0	

The data are plotted in figure 1, in which the abscissas represent the gradient solutions and the ordinates represent the average regeneration for each solution.

From table 1 and figure 1, it will be observed that *Cassiopea* lived in solutions in which the salt content varied from a lower limit of 40 to an upper limit of 153 per cent. In the extreme solutions the medusæ decreased in size, ceased to pulsate, and the organs degenerated. This occurred in the 40 and later in the 45 per cent as well as the 153 per cent solutions.

Within these limits the medusæ lived and pulsated, but not all regenerated. No regeneration occurred in solutions containing 50 per cent or less sea-water, nor on the other hand was there any regeneration in solutions 133 or more per cent, with one exception to be noted later. Though regeneration occurred within these limitations, the character of the resulting regenerated arms was not the same. Normal pyramid-shaped arms with characteristic mouths and oral filaments were developed in the median solutions only, *i. e.*, 75 to 105 per cent inclusive. In the 70 per cent solution the mouths were considerably reduced in number, while the number of oral filaments was scarcely affected. In the 65 per cent solution there were very few mouths differentiated. In the 60 and 55 per cent solutions practically no mouths were formed and the oral filaments tended to clump together. Similarly, beginning with the 111 per cent solution, of the concentrated series, the arms were increasingly atypic. In this solution some arms were normal, others clumped as in the diluted solutions. In the 117 per cent solution, fewer mouths were differentiated and greater crowding of the oral filaments occurred. In the 125 per cent solution no mouths and only very few filaments were differentiated. It is then clear that while regeneration took place in a wide range of solutions, normal regeneration occurred within much narrower limits, namely, 75 to 105 per cent solutions.

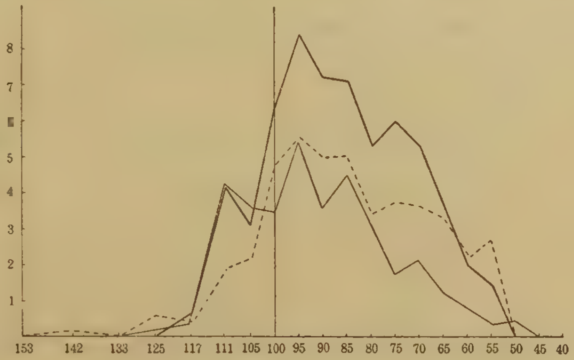

Fig. 1.—Thin line represents regeneration after 14 days: thick line 24 days; broken line 30 days.

Corroborative results were obtained from measurement of regenerated arms. Figure 1 and table 1 clearly show that when the average regeneration in sea-water is taken as the norm, then the average regeneration in 111 to 80 per cent solutions is norm or supernorm, varying from 3.4 to 5.4 mm. On either side of these limits there is a rapid decline, sharper in the more concentrated solutions, far less rapid in the more dilute series. For example, the average regeneration in the 117 per cent solution is less than in the 50 per cent solution.

The second series of measurements was taken 10 days after the first, *i. e.*, 24 days after removal of the arms. During these 10 days practically no regeneration occurred in the concentrated solutions, while retrogression set in. In all the dilute series, however, considerable regeneration occurred. This is seen in figure 1. Perhaps the facts are made clearer by table 2, which gives the difference in the average regeneration between the fourteenth and twenty-fourth days. Table 2 shows that in the concentrated solutions, 105 to 125 per cent, there was no additional regeneration; in fact, three out of four solutions showed a decrease and in one solution the increase was only 0.63 mm. In all the dilute solutions, however, there was an increase, in sea-water 3.03 mm., in 95 per cent solution 2.96 mm., 90 per cent solution 2.63 mm., etc. It should be noted that the increment was unequal, a greater relative and absolute increase occurring not in the optimum solutions, but in 75, 70, and 65 per cent solutions.

TABLE 2.—*Extent of regeneration during the second period, i. e., between the fourteenth and twenty-fourth days after amputation.*

Solutions.	Average gain or loss between 14th and 24th day.
	mm.
1.33	0
1.25	−0.33
1.17	+0.63
1.11	−0.09
1.05	−0.72
1.00 norm	+3.03
0.95	+2.96
0.90	+2.63
0.85	+2.59
0.80	+2.31
0.75	+4.22
0.70	+3.20
0.65	+2.39
0.60	+1.19
0.55	+1.14
0.50	0

In the third set of observations, made on the thirtieth day, the measurements were not taken from the amputated level, for at this stage of development it was difficult to tell with exactness where that level was. The last measurements were made from the end of the knob-like mass at the distal end of the amputated arms, and the measurements in table 1, therefore, appear smaller than the corresponding measurements for the preceding periods.

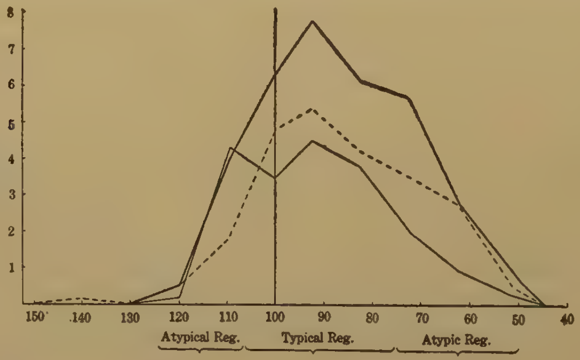

FIG. 2.—Thin line represents regenerating after 14 days; thick line after 24 days; broken line after 30 days.

The character of the curve is essentially the same as the 24-day curve, differing from it in the same way that it differs from the 14-day curve. It is seen that the curves for 105 to 75 per cent solutions are almost parallel. Beyond these limits the curves diverge. In the 75 to 55 per cent solutions there has been either an absolute or relatively greater increment than in the solutions approaching the norm. In the hypertonic solution there is no increase, the curves for the three periods are essentially parallel and overlap.

TABLE 3.

Per cent of sea-water in solution.	Average regeneration for 30 days.
	mm.
1.50	0
1.40	0.13
1.30	0
1.20	0.50
1.10	1.91
1.00	4.81
0.90	5.34
0.80	4.25
0.70	3.68
0.60	2.77
0.50	1.43
0.40	0

All these facts are made extremely clear after reducing some of the irregularities of the curves, by grouping the results on a basis of 10 per cent changes in salinity instead of 5 per cent, as shown in figure 2.

Table 3 shows the results of table 1 grouped on a basis of 10 per cent changes in salinity, instead of 5 per cent differences.

In the preceding tables and curves the fact was ignored that in certain solutions an arm was replaced by two arms. The supernumerary arms appeared in the median solutions, *viz*, 111 to 85 per cent, with a few desultory ones in more dilute solutions. The greatest number occurred in the 95 per cent solution, fewer in the 90 and 85 per cent, a sharp drop in 105 per cent and 111 per cent solutions, as shown in table 4.

Table 4 gives the number of supernumerary arms in each solution and shows that they tend to appear in optimum dilute solutions.

TABLE 4.

Per cent of sea-water in solution.	No. of arms removed.	No. of double arms regenerated.	Per cent of double arms.	Per cent of sea-water in solution.	No. of arms removed.	No. of double arms regenerated.	Per cent of double arms.
1.53	15	0	0	85	15	4	26
1.42	15	0	0	80	13	0	0
1.33	15	0	0	75	15	0	0
1.25	14	0	0	70	15	0	0
1.17	15	0	0	65	15	2	13
111	15	1	7	60	14	0	0
105	14	1	7	55	15	1	7
100	43	5	11	50	15	0	0
95	15	5	33	45	15	0	0
90	15	4	26	40	15	0	0

When the supernumerary arms are included in table 1, it is found that the highest part of the curve, 85 to 111 per cent, is augmented still further. The maximum augmentation occurs in 85 to 95 per cent solutions, and only a moderate increment occurs in sea-water.

Whether we consider only the regeneration of the supernumerary arms or of single arms, or normal as opposed to abnormal regeneration, or the variation in growth at different intervals, or all of these combined, it is clear that the maximum regeneration in *Cassiopea* occurs in sea-water diluted to 95, 90, and 85 per cent.

There are three sets of records complete enough to permit comparison, namely, Loeb's work on *Tubularia*,[1] Goldfarb on *Eudendrium*,[2] and the present contribution on *Cassiopea*.

Loeb's data are based upon the regeneration of 65 pieces of *Tubularia* stems over a period of 8 days. The work was done at Serino where the sea-water is estimated on the basis of Forchhammer's calculation to contain 3.8 per cent salts or more correctly 38.364 per kilogram. Loeb used 10 grades of solutions and plotted his results as shown in figure 3.

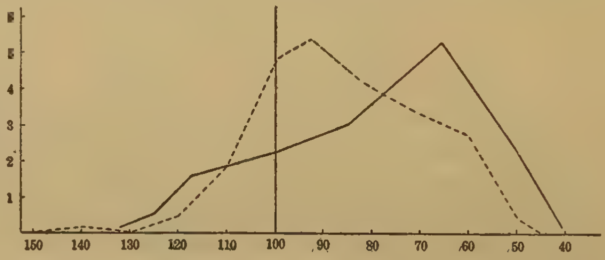

FIG. 3.—Thick line is Loeb's curve for *Tubularia;* broken line is curve for *Cassiopea* after 30 day interval.

The 323 *Cassiopea* arms observed at 14, 24, and 30 day intervals in sea-water whose salinity, though not definitely known, is calculated[3] to be 3.549 per cent. The medusæ were placed in 20 graded solutions. The results differ from Loeb's in at least four respects:

1. The salinity limits are different, *i. e.*, *Tubularia* regenerated in more dilute solutions than *Cassiopea*, though the extreme concentration is probably the same for both.

2. Maximum regeneration occurs at different salinities, 65 per cent sea-water for *Tubularia*, 95 per cent for *Cassiopea*.

3. There is a greater range of supernormal regeneration in *Tubularia* *i. e.*, 100 to 50 per cent salinity, while the range for *Cassiopea* is limited to 15 per cent, *i. e.*, from norm to 85.

4. The character of the curves is reversed. For *Tubularia* there is a graded increased regeneration with increasing dilution from 133 to 65 per cent; beyond this limit there is a sudden drop to complete inhibition in 40 per cent. The *Cassiopea* curve on the other hand rises rapidly to 95 per cent, beyond which there is a very gradual decrease (fig. 3).

The third set of observations were those on the compound hydroid *Eudendrium ramosum*. The results of one series, based on 953 stems, are plotted in figure 4, and when compared with *Tubularia* and *Cassiopea* show the following:

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[1] Loeb, J. Organization and growth. Wurtzburg. 1891.

[2] Goldfarb, A. J. Factors in the regeneration of a compound hydroid, *Eudendrium ramosum*. Jour. Exper. Zool., vol. 4, p. 43. 1907.

[3] Clarke, F. W. The data of geochemistry. Bull. 491, U. S. Geol. Survey. 1911.

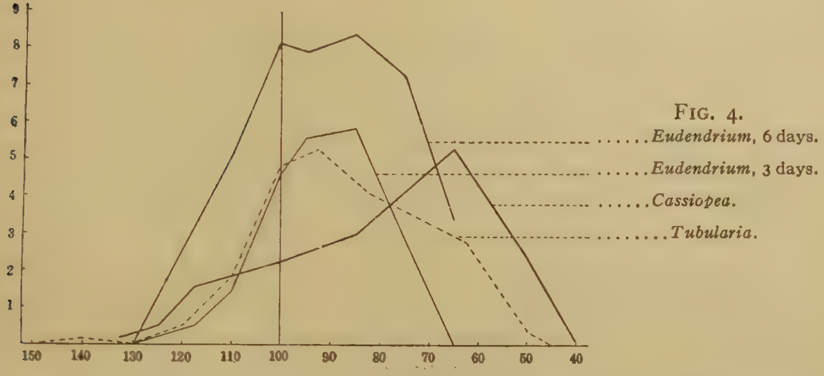

FIG. 4.

1. Regeneration takes place in a more limited variation from the norm than either *Tubularia* or *Cassiopea*, *i. e.*, the maximum salinity in which regeneration takes place is 120, the minimum is 60.

2. Maximum regeneration is intermediate, 65 per cent solution for *Tubularia*, 85 for *Eudendrium*, and 95 for *Cassiopea*.

3. Normal or supernormal regeneration in *Tubularia* occurs over a wide range of solutions, from norm to 50 per cent; in *Eudendrium* it is intermediate, 100 to 80 per cent; in *Cassiopea* most limited, 100 to 85 per cent.

4. The nature of the curves varies. *Tubularia* and *Cassiopea* have asymmetrical curves, *Eudendrium* almost symmetrical, *i. e.*, beyond the optimum solution, increased dilution or concentration is equally injurious.

Two sets of observations after 3 and 6 day intervals afforded the opportunity of comparing these with corresponding sets of observations on *Cassiopea*. The regeneration in *Eudendrium* is given in table 5.

TABLE 5.

Per cent of sea-water in solution.	Number of polyps regenerated in—		
	First 3 days.	Second 3 days.	Total, 6 days.
130	0	0	0
117	5	27	32
111	14	37	51
100 (norm)	46	36	82
95	56	23	79
85	62	22	84
75	28	45	73
65	0	34	34

It is apparent that the same phenomenon observed in *Cassiopea* occurred in *Eudendrium*, namely, that with increased intervals there is a greater relative regeneration, not in the optimum nor hypertonic solutions, but in the more dilute solutions, such as 75 and 65 solutions. It is very clear, both from *Cassiopea* and *Eudendrium*, that such changes in the sub-optimum solutions do not and can not alter the essential character of the curves sufficiently to make it analogous with Loeb's. The differences in the behavior of the three organisms is made easier for comparison by table 6, which gives the results of changes in salinity upon the three organisms studied by Loeb and Goldfarb.

TABLE 6.

	Maximum dilution.	Maximum concentration.	Optimum solution.	Regeneration occurs in—	Beyond optimum on concentrated side of curve.	Beyond optimum on diluted side of curve.	Salinity.	Curve.
	p. ct.	p. ct.	p. ct.	p. ct.				
Tubularia . . .	40	133	65	100 to 50	Less injurious.	Most injurious.	3.83	Asymmetrical.
Cassiopea . . .	50	133	95	100 85	Most injurious.	Least injurious.	3.54	Less asymmetrical.
Eudendrium .	60	125	85	100 80	Equally injurious.	Equally injurious.	3.29	Symmetrical.

In these three organisms the increasing dilution of sea-water, with the consequent intake of water into the organism, makes for a more rapid and greater growth. Beyond an optimum dilution, any further dilution retards and ultimately inhibits regeneration. Concentration of sea-water, in all cases, makes for an expulsion of water from the organism, with a concomitant retardation, decreased regeneration, and finally no regeneration whatsoever. Beyond these fundamental similarities, there is little or no agreement.

Though there appears to be little or no similarity in the behavior of these three organisms, on further examination it becomes quite clear that *Eudendrium* and *Cassiopea* are in essential agreement, both differing in a striking manner from Loeb's results on *Tubularia.*

Eudendrium and *Cassiopea* agree in the following respects: (1) maximum regeneration occurs in solutions that are hypotonic in a relatively small degree, *i. e.*, 95 and 85 per cent respectively; (2) regeneration in sea-water is equaled or exceeded in a narrow range of hypotonic solutions, *i. e.*, 100 to 85 and 80 per cent, respectively; (3) at any interval after amputation, the graphs show a *sharp* decline in the amount regenerated in increasingly hypertonic solutions; a maximum regeneration of not more than 60 per cent beyond the norm, *i. e.*, 60 per cent in *Cassiopea*, 35 per cent in *Eudendrium;* a *gradual* followed by a more rapid drop in the curve, in solutions more hypotonic than these optimum solutions.

It is in these very points that both these curves differ from Loeb's work on *Tubularia*, as follows: The maximum regeneration occurred in a markedly greater hypotonic solution, *i. e.*, 65 per cent; regeneration in sea-water is equaled or exceeded in a considerably wider range of diluted solutions, *i. e.*, 100 to 50 per cent solutions inclusive; the curve is completely reversed, the gradual increment and the sharp decline appearing on sides opposite to that in either *Eudendrium* or *Cassiopea;* the mode is nearer the extreme dilution; and the maximum regeneration is far greater (120 per cent) than in either of the other two organisms.

It is estimated that the maximum salinity of the sea-water at Woods Hole, Massachusetts, is 3.29,[1] for the Dry Tortugas 3.54 per cent.[2] It might be urged that a definite relation obtains between the density of the sea and the regeneration of the contained organism, subjected to a graded series of densities, for the increased density of the sea-water at the Dry Tortugas is paralleled by each of the changes already cited in table 6; but when *Tubularia* of Serino Bay, whose density is 3.83, is taken into consideration, no correlation between density and the character of the curve obtains, except in two particulars: (1) the greater the density of the sea, the greater the dilution in which regeneration will take place; (2) the greater the density of the sea the greater the regeneration in the hypotonic solutions, *i. e.*, *Eudendrium* Δ 3.29, maximum regeneration is 35 per cent above norm *Cassiopea*, Δ 3.54, 60 per cent, *Tubularia*, Δ 3.83, 120 per cent.

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[1] Bulletin of Bureau of Fisheries, vol. 31. 1911.
[2] Clarke, F. W. The Data of Geochemistry. Bull. 491, U. S. Geol. Survey. 1911.

Subject to a more accurate analysis of the density of the water in the moat at Fort Jefferson and at Serino Bay, these conclusions must be considered tentative only. The fact remains, however, that Loeb's limited data are not in accord with the results obtained in two different organisms from two different localities whose sea-water was of different densities. It is also clear that the more detailed and more numerous data upon *Eudendrium* and *Cassiopea* are in close agreement. And we are driven to the conclusion that either *Tubularia*, for some inexplicable reason, differs in its behavior from the other two cœlenterates, a view which is very improbable, or that the insufficient data upon *Tubularia* has misled the author. The close similarity of *Eudendrium* and *Cassiopea* and the corroborative results of A. G. Mayer upon the effects of changes in density upon nerve-conduction leads me to believe that the results here given may be taken as indicative of the gross effects, without any effort to segregate the osmotic from the ionic influences, upon the regeneration of these organisms.

SUMMARY.

The object of this investigation was to ascertain to what extent changes in salinity affected an organism, *Cassiopea xamachana*, normally subject to relatively great variation in the concentration of the sea-water, and to compare the results with those of the hydroid *Eudendrium* and the hydroid *Tubularia*.

Considerable attention was paid to render the following variable factors uniform for the series: Size of medusæ; volume, surface, and depth of the solutions; extent of injury; level of amputation; temperature; crowding.

Injurious or other variable factors were guarded against: (1) by aerating and changing all solutions daily; (2) by diluting with water containing known amount of sea-salts; (3) by concentrating the sea-water very slowly; (4) by examining the 323 regenerating arms at intervals of 14, 24, and 30 days.

Cassiopea lived in solutions ranging from 40 to 153 per cent sea-water solutions.

Regeneration occurred in solutions containing 50 to 133 per cent sea-water.

Normal regeneration of the arms occurred within much narrower range, namely, 75 to 105 per cent. Beyond these limits regeneration was atypic.

While the amount regenerated in sea-water was equaled or exceeded in solutions diluted to as much as 85 per cent, the optimum solution was 95 per cent sea-water. With increasing concentration, regeneration fell off rapidly; with increasing dilution the amount regenerated was diminished very slowly.

During the succeeding 10 and 16 days there was a relatively greater regeneration in solutions below the optimum, *i. e.*, in the 75 and 70 per cent, and the character of the curve was correspondingly altered.

The development of supernumerary arms likewise occurred most frequently in the optimum solutions, namely, 95 to 85 per cent; less frequently with increasing dilution or concentration.

When these results are compared with those of Loeb on *Tubularia* and with the writer's on *Eudendrium*, it is clear that in all the maximum regeneration does not take place in sea-water, but in sea-water diluted, and that with increasing concentration or dilution regeneration is retarded and diminished.

The character of the curves representing the influence of changes in salinity upon the regeneration of these organisms is strikingly different.

1. The most dilute solutions in which regeneration will take place is 40 per cent for *Tubularia*, 50 per cent for *Cassiopea*, and 60 per cent for *Eudendrium*.

2. The maximum regeneration occurs in 95 per cent solutions for *Cassiopea*, while it is 85 per cent for *Eudendrium* and 65 per cent for *Tubularia*.

3. The regeneration in sea-water is equaled or exceeded in solutions diluted as much as 13 per cent for *Cassiopea*, 20 per cent for *Eudendrium*, and 50 per cent for *Tubularia*.

4. The curve is most asymmetrical for *Tubularia*, less so for *Cassiopea*, and almost symmetrical for *Eudendrium*.

5. The *Eudendrium* behaves more like *Cassiopea;* both are strikingly different from *Tubularia*.

It is very evident that Loeb's curve is not representative of the behavior of marine organisms to changes in concentration, and it is altogether probable that such a curve can not be expressed in a simple curve based on two variables.

In the absence of information concerning the exact concentration of the sea-water, where each of these experiments was made, it is unprofitable to make extended generalizations.

V.

REGENERATION IN THE ANNELID WORM AMPHINOMA PACIFICA, AFTER REMOVAL OF THE CENTRAL NERVOUS SYSTEM.

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REGENERATION IN THE ANNELID WORM AMPHINOMA PACIFICA, AFTER REMOVAL OF THE CENTRAL NERVOUS SYSTEM.

By A. J. Goldfarb.

In a previous publication,[1] the writer found that the head of the earthworm *Lumbricus* was regenerated in the entire and permanent absence of the nerve-cord from the amputated region. Inasmuch as this was the first demonstrative experiment of a complete and perfect regeneration in the absence of the nerve-cord, it seemed extremely important to ascertain whether the phenomenon was peculiar to *Lumbricus*, and therefore of limited significance, or whether other annelid worms could likewise regenerate without the aid or presence of the nerve-cord. With this object in view, the marine annelid *Amphinoma pacifica* was chosen, because it lives so well in the laboratory, because large numbers could be readily procured, but especially, because it was sufficiently large and hardy to permit of the necessary operations.

There were two types of operations:

1. After narcotizing in a 5 per cent solution of alcohol in sea-water, the worms were amputated and the nerve-cord extracted. This was done by passing a fine forceps into the cœlomic cavity in such a manner that the nerve-cord lay between the tongs of the forceps, and at any desired position the forceps were firmly brought together and the contained nerve-cord was extracted with its ganglia and bases of the nerves. These could then be readily counted. I never succeeded in getting pieces so long as those in the earthworm, illustrated in my report.[1] I did succeed in extracting as many as nine consecutive ganglia. This method had the distinct advantage of causing very little injury to adjoining tissues. The importance of this fact will be later emphasized.

2. The second type of operation, used by Morgan[2] and modified by Nussbaum,[3] consisted in cutting a "window" from the ventral side near the amputated level. The parts so removed or injured included cuticle, muscle layers, nerve-cord, often the ventral blood-vessel, and the nephridia. A modification of the operation consisted in making a ventral incision, then

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[1] Goldfarb, A. J. The influence of the nervous system in regeneration. Jour. Exp. Zool., 7, 4, pp. 643–722. 1909.

[2] Morgan, T. H. Experimental studies of the internal factors of regeneration in the earthworm. Arch fur Entw. Mech., 14. 1902.

[3] Nussbaum, J. Beitrage zur Frage über die Abhängigkeit der Regeneration vom nervensystem bei *Nereis diversicolor*. Arch. f. Entw., vol. 25. 1908.

deflecting the muscle layers sufficiently to permit the removal of the exposed nerve-cord. The muscle walls were then replaced and ligatured.

There were 90 operations of the first type, 12 of the second, and 151 worms served as controls.

The worms were amputated at different levels and the nerve-cord removed from the anterior as well as the posterior parts. It is well known that the regeneration from the two exposed surfaces at certain levels may be quite different. The flatworm *Dendrocœlum lacteum*,[1] for example, regenerated a tail from the anterior piece and a head from the posterior piece, provided the amputation was not made more than one-third from the anterior end. Posterior to this level the head piece regenerated a tail, but the tail piece did not regenerate a head. In the earthworm *Lumbricus* the same phenomenon occurs. The differentiating level occurs at about the fifteenth segment.

Amphinoma differs from this type of regeneration in two respects: (1) the level at which the two cut surfaces can regenerate is much more posterior, *i. e.*, about the middle of the body; (2) instead of the regenerative power of the tail pieces being limited to certain narrowly prescribed levels, the reverse obtains, *i. e.*, head pieces can regenerate tails only in the posterior half or third of the worm, while tail pieces regenerate heads at practically all levels.

Table 1 gives some details of the regeneration in the head pieces of control worms, *i. e.*, level amputated, the number that regenerated a tail, etc. It is evident that no head piece regenerated when amputated less than one-eighth from the anterior end of the worm. Between the third and the middle of the body some of the pieces regenerated, others did not. All, however, regenerated posterior to the forty-fifth segment or middle of the body.

TABLE 1.

Level amputated.	No. of worms.	No. survived.	No. regenerated.	No. with no regeneration.
One-eighth from anterior end	9	8	0	8
Twenty-fifth segment	1	1	1	0
Thirty-fifth segment	1	1	1	0
One-third from anterior end	3	3	2	1
Middle of worm	5	5	2	3
Forty-fifth segment and farther posterior by actual count	9	9	9	0
Three-fourths from anterior end	4	4	4	0

In table 2 similar data are given for tail pieces of control worms. The data are arranged with respect to the level amputated and the number that did or did not regenerate. All of the tail pieces regenerated a head from the first quarter, the middle, and even from the distal quarter of the worm. Beyond this level a number of pieces did not regenerate. There were 9 such pieces that formed no head. Of these, 5 were small distal pieces 12 to 17 segments from the distal end, and probably too small to regenerate; in the other 4, I have no records of the exact level amputated.

TABLE 2.

Level amputated.	No. of pieces.	No. survived.	No. regenerated.	No. that did not regenerate.
One-fourth to middle	10	3	3	0
One-eighth to middle	22	13	13	0
One-eighth to seven-eighths	10	8	8	0
One-fourth to one-half	(?)	19	19	0
One-eighth to seven-eighths	18	17	10	7
One-eighth to seven-eighths	13	11	9	2

Tables 1 and 2 establish the fact that at anterior levels the head piece can not regenerate a tail, while the tail piece can regenerate its head. At posterior levels the reverse occurs: the tail piece can not regenerate a head, though the head piece can regenerate a tail. In the median levels both pieces may regenerate their respective missing parts. These preliminary facts concerning the regeneration of *Amphinoma*, after ordinary amputation, will aid in understanding the behavior of the head and tail pieces after the removal of the nerve-cord as already described. Of the 56 operated head pieces, 29 survived 25 to 30 days, as shown in table 3, which also gives the level amputated and the number that failed to regenerate.

TABLE 3.

	No.	No. regenerating a tail.
Operated head pieces	56	....
Survived	29	....
Amputated at one-eighth level	8	0
one-fourth level	3	0
one-third level	10	0
one-half level	7	0
three-fourths level	1	0

These operations were made at anterior levels before it was known that head pieces did not normally regenerate from these parts of the worm. Though regeneration took place readily at posterior levels of control worms, not one operated head piece developed a tail. The experiment appears to demonstrate that, in these instances at least, regeneration had been prevented by destroying the nerve-cord near the amputated level.

I have elsewhere suggested[1] that regeneration could be prevented and frequently was prevented by various means other than injury to the central nervous system. In the newt *Diemictylus*, the regeneration of the tail was prevented by plugging the cavity of the spinal column with paraffin. In the salamander Tornier[2] prevented the formation of the tail by operating the skin and muscle tissues in a manner to prevent their coordinate development. In *Amphinoma* regeneration was prevented not only by injury to the nerve-cord, but by removing the alimentary tract from five or more segments nearest the amputated level. In table 4 the anterior pieces with the alimentary tract so removed are arranged according to the level amputated.

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[1] Goldfarb, A. J. The influence of the nervous system in regeneration. Jour. Exp. Zool., vol. 7, 1909. The central nervous system in relation to the phenomenon of regeneration. Arch. f. Entw., vol. 32, 4. 1911.

[2] Tornier, G. Kamp der Gewebe im Regenerat bei Begunstigung der Hautregeneration. Arch. f. Entw., 22, 1906, p. 348.

TABLE 4.

Number of worms.	Number survived.	Level amputated.	Number regenerated.	Number not regenerated.
3	2	One-eighth	0	2
3	2	One-third	0	2
3	2	One-half	0	2
3	2	Three-fourths....	1	1

That regeneration was not prevented by injury to, or absence of, the nerve-cord can be demonstrated only by more direct proof. It must be shown (1) that regeneration did not take place, even though the amputated region was re-innervated by the regenerated nerve-cord; (2) that regeneration could take place without any trace of the nerve-cord in the region adjoining the amputated level. This proof was obtained by a detailed examination of serial longitudinal sections of posterior pieces. Table 5 includes the 46 posterior pieces from which the nerve-cord had been removed and which were subsequently preserved 25 to 30 days later.[1]

TABLE 5.

Amputated level.	Regenerated.	No regeneration.
One-eighth............	5	11
One-fourth............	0	3
One-third.............	0	3
One-half..............	2	3
Three-fourths..........	0	1

Out of 28 worms, 7 regenerated a distinct head; the other 21 did not regenerate in the same interval. Worm number 33 is typical of the 7 operated worms which regenerated their heads. The serial sections showed that the nerve-cord had been completely removed from the seven segments nearest the amputated level, agreeing with the record made at the time of operation. It was found that typical ganglia of the nerve-cord extended as far as the middle of the sixth segment from the amputated level, at which point the nerve-cord suddenly became narrow and extended in this very narrow, partially ganglionated condition into the fifth, fourth, third, second, and first segments, beyond the amputated level into the regenerated head. It continued dorsally and ended finally in a clearly differentiated supra-œsophageal ganglion. The details are identical with those described elsewhere[2] for *Lumbricus*. These worms afford no clue to the dependence or independence of regeneration upon the presence of the nerve-cord at the amputated level, nor of the manner in which the central nerve system was formed.

The second group of operated worms did not regenerate the head. I have arbitrarily designated a regenerated head one in which at least the stomadeum and "brain" were clearly differentiated. Worm number 32 may serve as an example of this group. In this worm the characteristic

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[1] The high mortality occurred chiefly in worms operated by the "window" method of removing the nerve-cord.
[2] Goldfarb, A. J., *vide*.

nerve-cord was present as far as the eighth segment from the amputated level. Beginning at the seventh segment the nerve-cord was very narrow and extended in this attenuated condition through the sixth, fifth, fourth, third, second, and first segments. The regenerated nerve-cord had not only reached the amputated level, but extended slightly beyond, into the proliferated embryonic tissues laid down at the cut end. A similar regeneration of the nerve-cord occurred in worms 13, 9, 45, etc., in which the nerve-cord regenerated two to seven or more segments, and in which the nerve-cord did not always reach or pass beyond the amputated level.

In the worms whose nerve-cords had regenerated to the amputated level, supplying this region with nervous and trophic stimuli, the lack of regeneration could not have been due to any lack of innervation.

In a third very small group the head regenerated without the presence of the nerve-cord at the amputated level. In worm No. 7, for example, the characteristic nerve-cord extended as far as the sixth segment from the amputated level. The regenerated nerve-cord was present in the fifth and part of the fourth segment. Beyond this region, *i. e.*, the fourth, the third, the second, and posterior half of the first segment, there was no trace of a nerve-cord. Close to the amputated level and slightly beyond it into the embryonic tissues, there was an unmistakable ganglionic mass, from which there extended posteriorly a short, narrow nerve-commissure. This supraœsophageal ganglion had been formed independently of the direct contact or stimulation from the regenerating nerve-cord.

Worm No. 34 affords a much better idea of the development of the newly formed head. The nerve-cord was intact as far as the sixth segment, from which point the regenerated nerve-cord extended through the fifth, fourth, and third segments. The next two segments were entirely free of traces of the nerve-cord. Beyond the amputated level there was a fairly large regenerated head with a clearly differentiated "brain," from which the nerve-commissures extended ventrally and posteriorly almost to the amputated level. These two worms clearly show that it is possible for a head to regenerate with the nerve-cord 2, 3, or more segments from the amputated level. They show that the development of the brain and commissures may secondarily unite with the regenerating nerve-cord, as was found to be the case in *Lumbricus*.

In view of the regeneration of the head in *Lumbricus*, in the absence of innervation from the nerve-cord, and in view of the corroborative results obtained in *Amphinoma*, it seems evident that regeneration can take place under these circumstances. It is very probable that the presence of the nerve-cord accelerated regeneration. It may also be that regeneration does not take place ordinarily until the amputated level has been innervated from the cells of the nerve-cord as urged by Nussbaum.[1] It is equally clear, however, that a head can be regenerated in at least two annelid worms without the aid or stimulus derived from the nerve-cord.

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[1] Nussbaum, Josef. Beitrag zur Frage uber die Abhangigkeit der Regeneration vom Nervensystem be *Nereis diversicolor*. Arch. f. Entw., vol. 25. 1908.

SUMMARY.

The marine annelid worm *Amphinoma pacifica* readily regenerated a head at all levels except the distal eighth of the worm.

The formation of a tail occurs only in the posterior half or third of the body.

Only in the median levels of the body can the two cut surfaces regenerate a head and a tail respectively.

Regeneration may be prevented by a severe injury, either to the digestive tract or to the central nerve system; the greater the injury the more likely will regeneration be inhibited.

Many pieces did not regenerate after removing the alimentary tract from five or more segments nearest the amputated level.

Many pieces, about one-third, failed to regenerate after removing the nerve-cord by the forceps, *i. e.*, with little injury to adjoining tissues.

All failed to regenerate after removing the nerve-cord by the "window" method, *i. e.*, with serious injuries to adjoining parts.

The operated worms were examined in serial sections. In one group a regenerated nerve-cord connected the regenerated "brain" and commissures with the old intact nerve-cord.

In a second group the regenerated nerve-cord approached and in many instances reached the amputated level, yet no head was formed.

In a third group, the nerve-cord had regenerated, but several segments nearest the amputated end were yet without any nerve-cord or ganglia. These worms nevertheless had regenerated a head with its typical brain and nerve-commissures.

The last group completes the demonstration that *Amphinoma* as well as *Lumbricus* can regenerate without the contact of, or stimulation from, the nerve-cord or central nervous system.

VI.

EXPERIMENTALLY FUSED LARVÆ OF ECHINODERMS WITH SPECIAL REFERENCE TO THEIR SKELETONS.

BY A. J. GOLDFARB,
Of the College of the City of New York.

Fifteen text-figures.

EXPERIMENTALLY FUSED LARVÆ OF ECHINODERMS WITH SPECIAL REFERENCE TO THEIR SKELETONS.

By A. J. Goldfarb.

The early work of Loeb, Morgan, and Herbst on the production of multiple embryos from a single egg suggested the reverse experiment of grafting or reuniting several fertilized eggs into one embryo. This important experiment was first performed by Driesch, who succeeded in grafting together two eggs in about 0.04 per cent of the culture. With the same technique he subsequently grafted the eggs in other species of echinoderms and succeeded in keeping such grafted groups alive until they had developed into the larval or pluteus stage. He found that these larvæ, fell into several distinct types, as follows: true twins, twins with a common blastocœle, twins with a reciprocal influence on growth, fusion with a partially double archenteron, perfect fusion with a single set of organs, and single body with a second parasitic archenteron.

In 1912, the writer repeated these experiments with the American form *Arbacia punctulata* and succeeded only after slightly modifying Driesch's method. In the best cultures about 40 per cent were fused in groups of 2, 3, 4 or more eggs. These fused groups were preserved at different stages of development for further detailed study, but sufficient precautions had not been taken to guard against the solution of the very delicate skeletal structures of the larvæ. Consequently the early stages only were available for detailed and complete study. Such studies corroborated and extended the results of Driesch.

Subsequently, in the performance of other experiments, it was discovered that eggs could be agglutinated and fused quite as readily by a very different method, which was not only simpler but free of certain objections that might be urged against previously known methods. The new method consisted in using an isotonic or slightly hypotonic NaCl solution diluted with varying quantities of sea-water.

NEW METHOD OF FUSING EMBRYOS.

The best results were obtained by the following procedure: The eggs of *Toxopneustes* were artificially fertilized, and 15 to 20 minutes later approximately equal quantities were placed in solutions made up of 70 to 80 parts of 5/8 m. NaCl (isotonic with sea-water at the Tortugas) and 30 to 20 parts of sea-water. After 5 to 8 hours in these solutions the eggs were transferred to fresh sea-water. In the best cultures the number of agglutinations and subsequent fusions were as numerous as in the best cultures of *Arbacia* with the modified Driesch's method.

EARLY DEVELOPMENT.

Inasmuch as I have elsewhere described in detail the early developmental changes in agglutinated and fused embryos of *Arbacia* and since the changes in *Toxopneustes* are essentially identical with those in *Arbacia*, I will only supplement the previous account by stating briefly at what stage and under what circumstances agglutination occurred after treatment with the NaCl solutions.

The addition of an isotonic NaCl solution to sea-water involves a dilution of the other sea salts and a concentration of the NaCl. The excess NaCl stimulates the eggs to develop more rapidly, provided that the solution does not contain more than 50 per cent of the NaCl solution. In solutions containing 50 to 60 per cent NaCl solution, the excess NaCl seems to be neutral, but beyond this quantity with 90 or more per cent development was distinctly retarded and atypic. That the eggs in the NaCl solutions absorbed water was evidenced by the distinct increase in size over the corresponding controls, and by the diminution in the space between the egg and the fertilization membrane. In many instances the fertilization membrane burst and a part of the protoplasm of one or more of the blastomeres protruded from the fertilization membrane, while in other eggs this membrane was completely thrown off.

The differential rate of development in the NaCl solutions closely paralleled the differential rate of regeneration in varying concentrations of sea-water. This suggested that the agglutinations might be due to a graded intake of water. But no agglutinations occurred in ordinary diluted or concentrated sea-water, or in sea-water to which $MgSo_4$, K, Ca, or Li salts had been added, or in sea-water to which various anesthetics had been added, or with eggs whose fertilization membranes had been removed, or in sea-water rendered alkaline. The NaCl in the above experiments must have affected the eggs in such a manner as to gelatinize their outer surfaces, and under these circumstances, when two or more eggs whose fertilization membranes were ruptured came in contact, they were agglutinated. Some of these agglutinated eggs subsequently separated, others remained agglutinated, developing into more or less perfect twins, while others fused together. I have never observed fusion during the egg stage, as reported by Haan. The fusion in both *Arbacia* and *Toxopneustes* occurred and was limited to the blastula and gastrula stages, as described in a previous publication. In these studies and in those of other workers in this field attention has been chiefly directed to the fusion of the ectodermal walls of the two embryos and to the fusion of their archentera. The skeleton seems to have been overlooked. Curiously enough the changes in the skeletons of two fusing larvæ indicate most clearly the nature of the regulatory phenomena during this process. This paper will deal with the consideration of the changes in the skeletal structures in fusing larvæ.

SINGLE PLUTEUS LARVA.

Except where specifically mentioned to the contrary the drawings were made with the camera lucida and drawn to the same scale. Figure 1 is a lateral view of a single larva fully devloped. The figure represents the larva somewhat foreshortened, but with all of the parts, particularly the skeletal structures, outlined. Within the body of the larva are two ventral body rods, one on each side of the body. From these rods two long bars extend into the ventral arms. At right angles to these, and arising from the union of the ventral body rods and the ventral arm rods, are two ventro-dorsal connectives, which in turn bifurcate, one branch extending into the dorsal arm and the other aborally into the body to form the dorsal body rod. From the aboral end of the ventral body rods are two usually bifurcated aboral branches. Finally, there are two ventral branches between the ventral arms, but these are not shown in the figure. These bars are easily recognized from one another in both the single and fused larvæ, and they afford a definite and measurable index of the changes during the fusion of the larvæ.

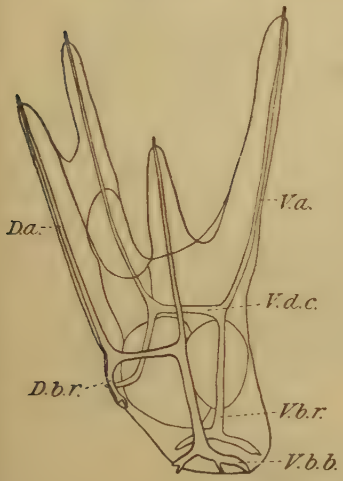

FIG. 1.

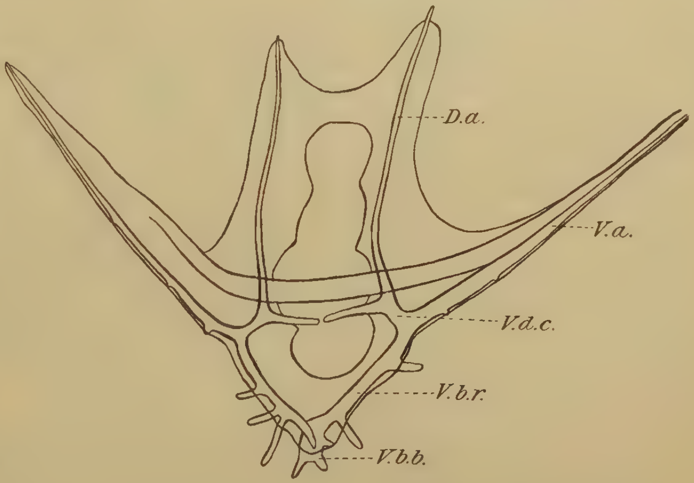

FIG. 2.

Figure 2 is another single larva drawn under greater magnification, It shows, from a foreshortened ventral view, the same characteristic parts of the skeleton, and also illustrates the small knobs and processes on the ventral rods, which are sometimes not reproduced in the drawings. Figures 3 and 4 are enlarged to the same scale as figure 2 and represent the ventral and lateral views of the skeleton. Figure 4 affords a particularly good example of the spinous ventral body rods.

FUSED PLUTEI.

Agglutinated or "true twin" larvæ are of no special significance for my present purpose, for each is perfectly developed and no reciprocal influence occurred. Figures 1 to 3 correctly represent any such agglutinated larva. It is only in fused larvæ, *i. e.*, larvæ with a common body cavity and a common and continuous ectodermal wall, that reciprocal changes are observed.

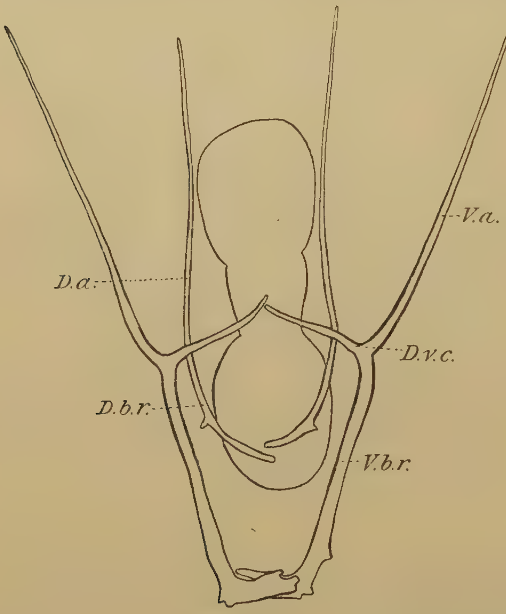

FIG. 3.

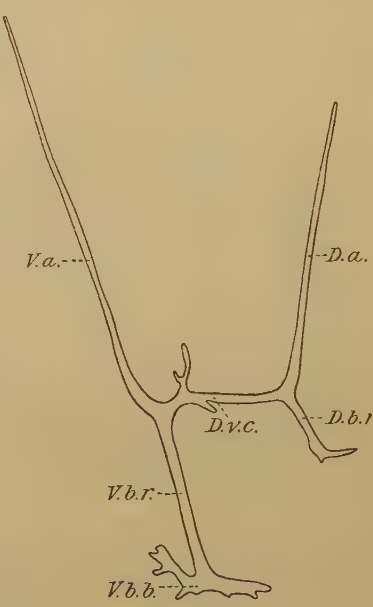

FIG. 4.

In the following drawings, for the sake of simplicity, I have not always indicated the whole skeleton when such omitted portions are normal and when the symmetrical right or left side is fully represented. Figure 5 is a partial fusion of two larvæ with a common body cavity and a continuous body wall. Each larva is completely differentiated and normal. The archentera are complete and independent, though they appear to be superimposed in this particular view of the fusion. The skeletons are typical and the parts are complete and characteristic of the normal single larva. On closer examination it is found that certain parts have not developed or have not reached their full size, such as the left dorsal rods of the right or A pluteus, and the left dorsal arm of the left or B pluteus. Associated with this suppression or retardation of the dorsal rods there is a compensatory growth of the left dorsal arm of the B pluteus. This supernumerary arm bifurcates, one branch extending into the right pluteus, the other branch into the left pluteus. And though the skeletal rods of the two larvæ are in close proximity and overlap in several places, there is no trace of a fusion of the rods. This suppression of the dorsal rods and the associated compensatory growth of other parts of the skeleton were observed in varying degrees of complexity in all or nearly all the fused larvæ.

The two plutei of figure 6 are fused more completely together than the preceding pair of plutei. The two larvæ are perfect and the parts are typical and independent, except the body wall, which is common to both. In the right or A pluteus each of the skeletal parts is differentiated and normal. In the left or B pluteus the ventral body rods are incomplete in so far as they do not possess their aboral bifurcated branches, and one of the ventral arm rods is abbreviated. The B pluteus in this fusion is incomplete, not with respect to the dorsal rods, as in the previous example, but in the aboral branches of the ventral body rods.

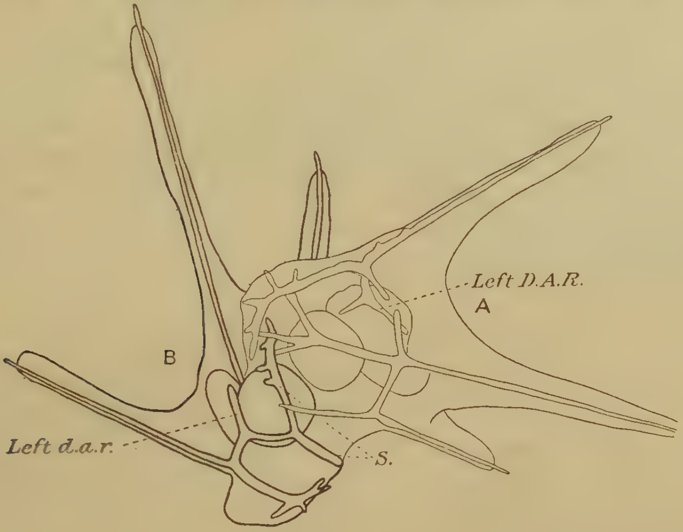

FIG. 5.

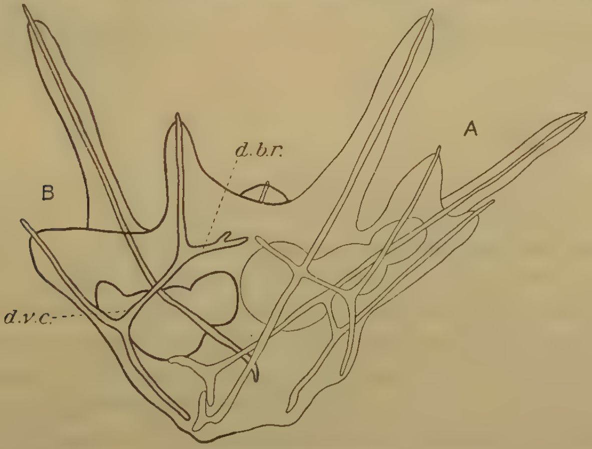

FIG. 6.

It is well known that in the development of the skeleton the mesenchyme cells differentiate two triradiate spicules, one on each side of the body. The bars of the triradiate spicules develop unequally. The ventral body rod, the ventral arm rod, and the dorso-ventral connective are formed from the outgrowth of the original three bars. Subsequently the dorsal body rod and the dorsal arm rod are differentiated, and finally the aboral branches of the ventral body rods. In the two examples of fused larvæ just described, it was found that, when skeletal parts are suppressed, such suppression is limited to the parts last to be differentiated, namely one or both dorsal rods and the aboral branches of the ventral body rods. That this suppression was permanent can readily be demonstrated. It will be recalled that all parts of a pluteus are fully formed within 72 hours, yet these fused plutei, after a period of 4 to 7 days, had not differentiated their missing rods.

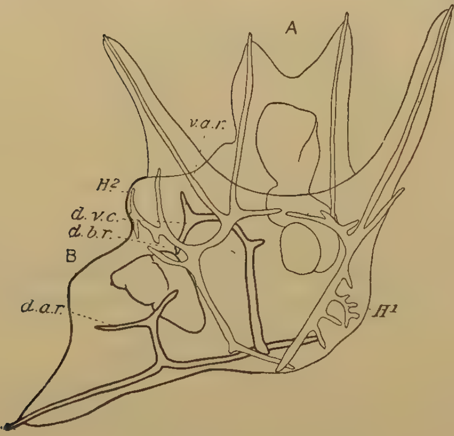

FIG. 7A.

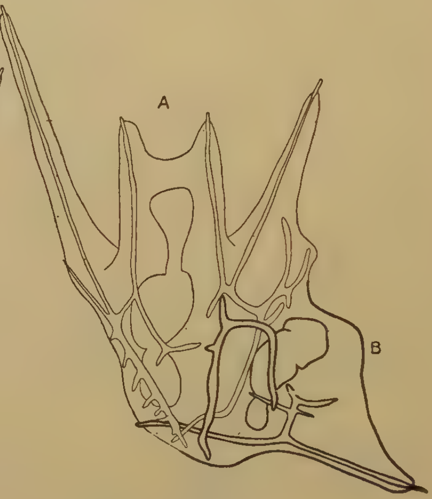

FIG. 7B.

The last fusion does not offer such clear evidence of compensatory growth as the preceeding fusion; it is probably limited to the extremely elongated dorso-ventral connectives of the B pluteus.

In the last two instances the fused plutei were equal or nearly equal, both in size and in the stage of development. In a large number of instances, however, the two plutei are unequal in both these respects and in varying degrees of inequality. For example, figures 7A and 7B are two views of the same pair of fused plutei, of which figure 7A is somewhat enlarged. In these figures the A pluteus is clearly larger and more completely differentiated than its mate; the four arms, the shape of the body, and the archenteron are completely differentiated and normal. The B pluteus appears to have but one abbreviated arm and a diminutive archenteron.

The skeletons show very clearly the unequal development of the two plutei. The A pluteus contains all the skeletal parts of the normal pluteus, and their shape and size and relative positions are essentially normal. The skeleton of the B pluteus is far from complete. It contains the two ventral body rods and two dorso-ventral connectives which are quite normal, but the two dorsal body rods, one of the ventral arm rods, and one dorsal arm rod are very much reduced in size, and there is no trace at all of the aboral branches. The compensatory growths of the skeletons in this fusion are very striking indeed. They are altogether limited to the A pluteus and confined to processes on the right ventral body rod at H^1 and on the left ventral body rod at H^2, where the outgrowth is much branched and extends into the B pluteus. The formation of these hypertrophied and supernumerary bars is explicable on the assumption that when the mesenchyme cells were prevented from developing within *its* own larva they migrated into the adjoining or A larva and there formed the additional skeleton at or near the center of active skeleton building, namely, the region of the union of ventral rods and dorso-ventral connectives.

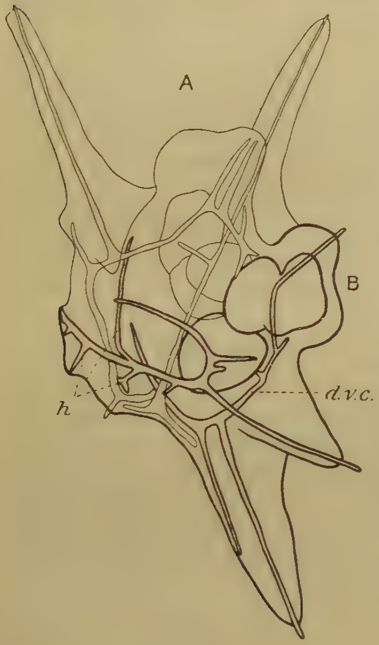

FIG. 8.

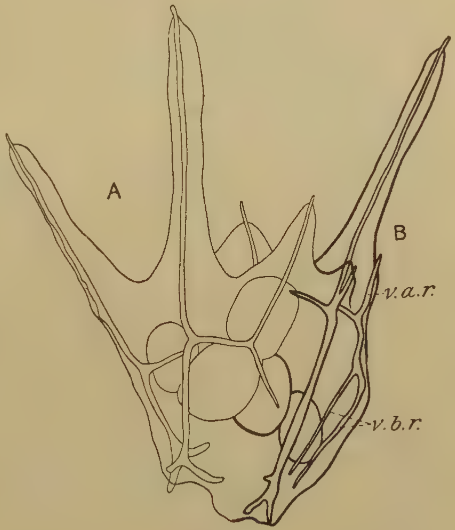

FIG. 9.

Figure 7B is a dorsal view of the same pair of fused plutei, which shows more clearly some of the points referred to.

Figure 8 represents two plutei much more completely fused into one body. The dominant or A pluteus is typical and complete; the suppressed or B pluteus is smaller and incomplete, and this inequality is shown particularly well in the skeletons. It will be observed that the B pluteus is deficient in these skeletal parts that were missing or abbreviated in the previous examples, namely, the dorsal arm rods and the aboral ventral branches. The other parts are perfectly differentiated. If the hypothesis of the migration of the mesenchyme cells from the suppressed to the other larva is correct, one would expect in this fusion correspondingly smaller compensatory growth to have taken place; this is found to be the case, namely additional processes on the ventral body rod shown at *h* and an elongated dorso-ventral connective.

A rather interesting instance of form equilibrium is seen in the A pluteus, where the left dorsal arm is absent and the right has formed an accessory arm rod and dorso-ventral connective. This fusion affords particularly strong evidence that the skeletons of the two plutei do not fuse in the ordinary acceptance of that term, for the overlapping of the parts is here very considerable.

In figure 9, the inequality of the two fused plutei is very great. The A pluteus is perfect in every detail, the B is very incomplete. In the first place, there is but one in place of four arms, and the gut is fused to the foregut of the A pluteus. The skeleton consists of two ventral body rods, only one of which possesses an aboral branch; two dorso-ventral connectives, and one large and one very small ventral arm rod. There is no trace of the dorsal arm or body rods. As in previous instances, such suppression of the skeleton is associated with compensatory growths, which, in this instance, is confined to the supernumerary ventral body rod and to a marked thickening of the other ventral body rod of the B pluteus.

The fusion shown in figures 10A (somewhat enlarged) and in 10B is particularly interesting. It resembles figure 9 in so far as the A pluteus is complete and true to type, while the B pluteus is very incomplete. The dominant pluteus has all the skeletal parts, the other (B) has but one ventral arm and an atypic archenteron. The skeleton of this pluteus consists of only three out of at least twelve parts, namely one ventral body rod, one ventral arm rod, and one dorso-ventral connective. Over three-quarters of the skeleton has not been differentiated.

The compensatory growth of the skeletal structures is to some degree commensurate with the degree of incompleteness or suppression. Close examination of figure 10 will show that the right dorso-ventral connective is not the normal single rod, but is much branched, and that from it a very long bar extends across the body of A into the body of B; it reaches almost as far as the ventral body rod of B, and might easily be mistaken for its symmetrical mate. Figure 10B affords another view of this fused larva and shows in a more striking manner some of the facts just referred to. In passing, it might be noticed that the three short rods derived from the dorso-ventral connectives of B are probably other supernumerary or compensatory outgrowths of the skeleton.

The extension of the large supernumerary (S) rod across one pluteus and into the other might be accounted for in one of two ways. Either it was formed originally as a ventral body rod in the B pluteus and secondarily united with the skeleton of the A larva at its dorso-ventral connective, or it was an hypertrophied dorso-ventral connective of A which grew beyond the limits of its own body into B. The matter can be settled conclusively only by continuous observation of the living fusion, which was not made in the case of this particular fusion. The preceding and certain of the following fusions make it altogether probable that a fusion of the two skeletons did not take place, but that the mesenchyme cells migrated from one part of the body to another, as in figures 5, 6, 8, and 9, or from one body into the other, as in figures 7 and 10. On comparing figures 7 and 10, it will be observed that the supernumerary rod in both originated in the same region, is much branched, extends from one larva into the other, differing only in the matter of length, and finally that they replace the suppressed parts of the skeleton of the weaker larva. The close similarity in these respects is significant and supports the view of a redistribution of the mesenchyme cells and the subsequent outgrowth of the hypertrophied rods into the second larva.

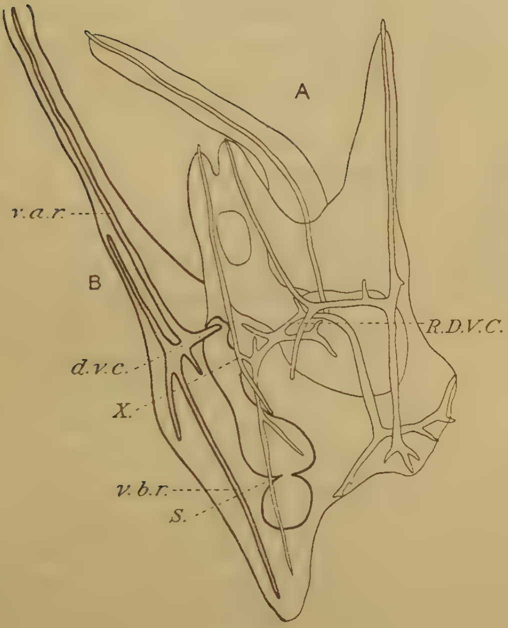

FIG. 10A.

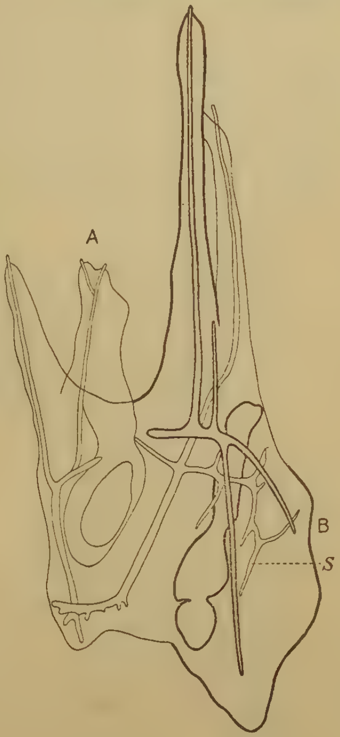

FIG. 10B.

Figure 11, which is somewhat enlarged, might be mistaken for a single larva with a supernumerary arm. On closer examination it will be found that there are clearly two larvæ fused very completely together. One of these, the dominant or A pluteus, has the characteristic four arms, perfect archenteron, etc. The other or B pluteus is represented externally by a single arm. This arm, however, contains a ventral and a dorsal arm rod united by a dorso-ventral connective, from which a characteristic ventral and a dorsal body rod extend into the common body cavity. The arm of B is really the right or left half of the B larva and if there be any doubt as to the dual character of figure 11, the diminutive archenteron in the B larva should at once set it aside.

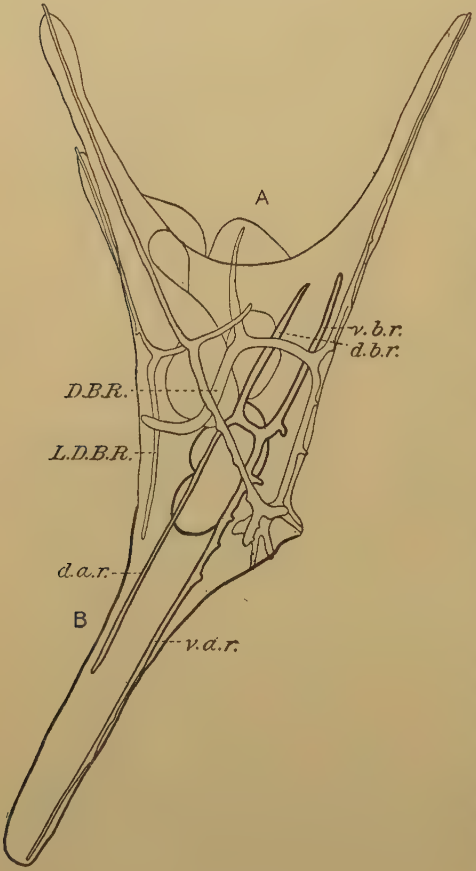

Fig. 11.

There are several instances of compensatory growths in the dominant larva. There is the hypertrophied dorso-ventral connective, and better still the dorsal body rod which is not merely considerably longer but extraordinarily thicker than in the controls, and finally an elongated left dorsal body rod.

It should again be noted that although there is considerable overlapping of adjoining skeletons there is no fusion at any point.

In the fusions of the larvæ so far described, the archentera were either independent or partially fused. In the following examples the two larvæ are more completely fused together, and there is but one archenteron, the one belonging to B having been either suppressed or fused completely in that of A.

Figure 12 is an example of a very complete fusion of two larvæ with but one archenteron. Detailed inspection shows that one larva is complete and perfect, while the other has but a short blunt arm as the external evidence of the second larva. Within this arm there is a normal and characteristic ventral body rod with a number of hypertrophied processes, a true dorso-ventral connective and an unusually thin ventral arm rod, and an accessory dorsal arm rod. In this larva the right or the left half of the larva has been differentiated, but less completely than the half larva of figure 11.

Figure 13 resembles the fusion shown in figure 12 in several essential points. Each fusion has but one archenteron, a dominant and perfect larva, and a very incomplete larva represented by a single broad arm, in which there is a ventral arm rod, a ventral body rod, and a dorso-ventral connective. In figure 13 there are two thin, straight bars—one long, the other very short, neither of which connects with either the B or the A skeletons. If it be assumed that retrogressive changes have taken place and that these have affected one or both ends of certain rods, we should expect the connections between rods to disappear, the distal ends to be shortened, and the bars themselves to be thinner than corresponding rods of control larvæ. All these results are found in figure 13 and the independent bars probably represent the former dorsal and ventral arm rods.

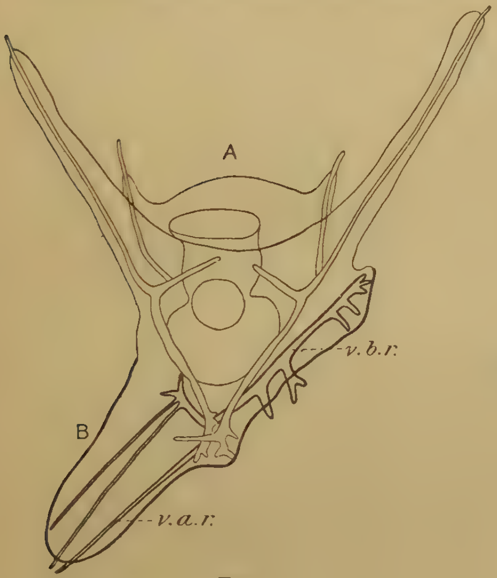

FIG. 12.

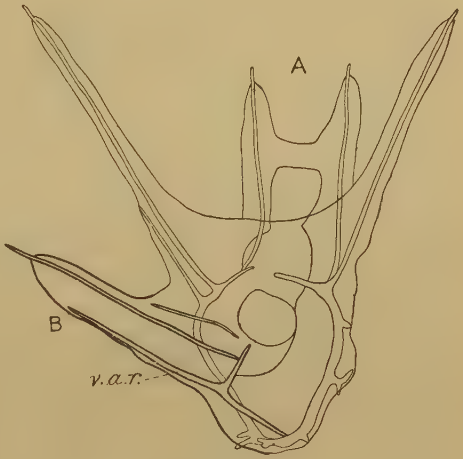

FIG. 13.

In figure 14 form equilibrium is more closely established. Larva B is reduced to a small, very blunt protuberance on the left side of the figure. It consists internally of a ventral body rod and its aboral branch, a very much dwarfed ventral arm rod, a dorso-ventral connective, a dorsal body rod, a supernumerary rod at s and a mere rudiment of the dorsal arm rod. In other words only *one half of one side of the skeleton has been formed* in this larva.

The gradual decrease in size and contents of the B larva, seen in figures 11 to 14 inclusive, reaches an extreme state in figure 15; and if it were not for the intermediate stages already described, figure 15 might easily be overlooked or mistaken for a single larva.

In figure 15 the A larva contains all the parts in their proper relative positions typical of the control larva. The gut is somewhat enlarged, indicating the incomplete fusion of the two archentera. The skeleton is single and perfect and to describe it is to describe the control larva. The B larva is represented only by a short, blunt arm containing a *single straight rod,* probably a ventral arm rod, which passes the ventral body rod of the A larva and ends blindly in the common body cavity. This single rod is less than one-twelfth of the complete skeleton whose parts have either been suppressed or disintegrated after their formation. If either or both of these processes were to continue beyond the stage shown in this figure the blunt arm would disappear with its contained single bar and all trace of the B larva would be gone. It would then be absolutely impossible to distinguish such a larva from the non-fused larvæ in the rest of the culture.

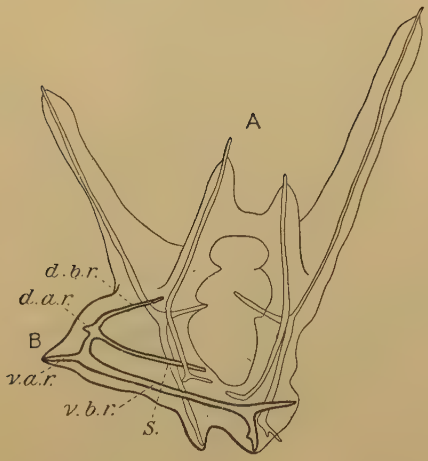

FIG. 14.

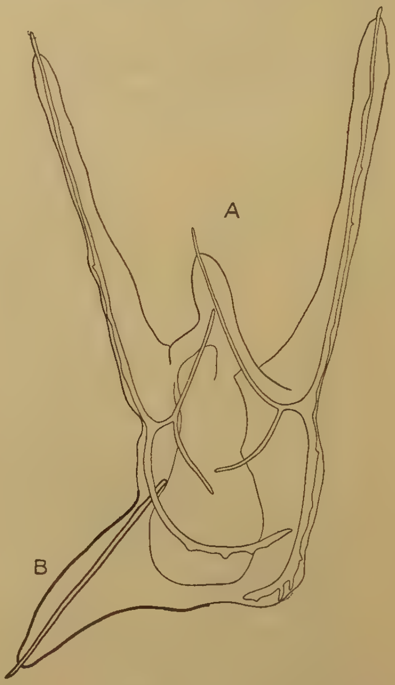

FIG. 15.

DISCUSSION.

In a previous publication I have shown that two or more blastulæ or gastrulæ derived from separately fertilized eggs, when united, tended to be remolded into a single gastrula somewhat larger than the controls. In this paper I have shown that this process of form regulation continued through the larval period, during which the tissues were completely differentiated. Such fusing larva tended to be remolded into a single one of normal size, but this tendency is conditioned by certain factors, of which the following are the most important:

(1) *Area of the agglutinated surfaces of the two blastulæ:* When the developing blastulæ are merely agglutinated by an inconsiderable area of their surfaces, each develops into a gastrula and a pluteus—complete, perfect,

and independent of the other except for the common surface of attachment. There is no reciprocal influence of one upon the other. When, however, the agglutinated surfaces extend over a large area the blastulæ tend to fuse, to have a common blastocœle, and the gastrulæ tend to approximate the form of a single embryo. It can not be stated definitely which is cause and which effect, whether greater common surface makes for more complete fusion or whether the internal forces making for fusion also affect the surfaces of the agglutinated embryos.

(2) *Mass of agglutinated or fused embryos:* When more than four or five eggs are agglutinated the disintegration of one or more of them at an early stage in their development brings about the disintegration of the entire cluster before they can fuse. When three blastulæ are agglutinated they may develop into three perfect and practically independent larvæ, or they may fuse together, under which circumstance the reciprocal influences are exceedingly complex and the resulting larva is quite atypic. For this reason I have omitted all consideration of such complex fusions and limited myself to fusions of two eggs, so that the regulative changes may be observed with the least number of disturbing factors.

(3) *Differential rate of development:* When two gastrula are fused and each has developed at an equal rate, twin larvæ like that shown in figures 5 and 6 result, in which case each larva is practically complete and equal in size, and has little or no influence upon the other. When two gastrula have not developed equally the conditions are then most favorable for form regulation.

At least two factors are involved in form regulation, namely, the suppression of structures and the disintegration of those already differentiated.

I have elsewhere shown, from the study of living fusions, that the archenteron of one of the fused gastrulæ may be entirely inhibited or may never develop beyond an early stage of its differentiation. Driesch and de Haan have also observed aborted or dwarfed archentera. I have shown in this paper that certain parts of the skeleton also may never be developed, that such suppressed parts are nearly always the last to be differentiated and confined to but one of the fused larvæ.

It has been urged by de Haan that the suppressed larva is a sick larva, that there is no regulation but that a union of a healthy and an unhealthy larva occurs. Under such circumstances we should expect the sick larva to be atypic and some relation between diminutive size and degree of irregularity in development. But the fused larvæ are quite perfect and often of normal size. It might be urged that only very sick larvæ would be atypic and would not complete their development. But how can one account for the lack of development of the right or the left sides of the skeleton on such an assumption? It would also be exceedingly difficult to account for the absence of the oral half of one side (fig. 14), particularly in the face of the fact that the skeletal parts that are differential *are characteristic and normal* and show no trace of irregularity or sickness.

Since the suppression of skeleton, arms, and archentera, etc., do not occur in control larvæ, it seems reasonable to suppose that the more rapidly developing larva influences the other in such a manner that the mesenchyme cells of the latter are prevented from completing the skeleton, and the degree of suppression seems to be related to the differential rate of development, the greater the difference the greater the suppression of the parts of the slower developing larva.

The second factor making for form equilibrium is the loss of parts that have already been differentiated. I have shown from the study of living gastrulæ that the archenteron in the slower developing gastrula in a fusion may be reduced in size and completely disappear. The skeletons may also actively degenerate in one of the fused larvæ. This degeneration is evidenced in the reduction in thickness of various rods, in the disintegration of one or both ends of rods, or both of these processes. As a result of the incomplete development of the skeleton and the subsequent disintegration of parts that have been formed the skeleton may ultimately consist of but a single or a few independent rods. And since the size of the body is so dependent upon the size of the skeleton the suppressed larva may decrease in size until it almost disappears, as shown in figure 15, and I believe may completely disappear.

What happens to the tissues of the degenerating larva? Are they absorbed as food by the dominant larva as nurse cells supply egg cells with nutriment; or are they disintegrated after the manner of certain grafts without any influence upon the host; or are they transferred and reconstructed within the body of the dominant larva? There is unmistakable evidence that two of these processes ordinarily take place, but in unequal degree and affecting different structures of the organism.

The transfer and rebuilding of the materials of one larva into the other are limited to the last differentiated tissues, namely, the skeleton. It was shown that when two fused larvæ developed unequally the dominant pluteus may prevent the completion or the development of parts of the skeleton; that under these circumstances the unused mesenchyme cells may migrate to other regions of the body or *into the body of the dominant larva* and there give rise to additional skeletal material, either by the thickening or elongation of the rods or by the growth of supernumerary processes and rods. The extent of such hypertrophied or supernumerary growths was commensurate with the suppression of skeletal parts in the other regions.

De Haan seems to have shown that two eggs may be completely fused into one somewhat larger than the control. Driesch, Goldfarb, and de Haan have demonstrated that blastulæ may be completely fused together into a single larger blastula. The evidence is not so conclusive but seems to point to a complete fusion of gastrulæ and the enlargement of the gut as well as the body.

Beyond this stage in the differentiation of the tissues a complete fusion of the organs of the two larvæ does not take place, at least in *Toxopneustes* *variegatus.* The skeleton under no circumstance fuses in the two individuals and there is no evidence of an enlargement of the larva to form the "einheits pluteus" or completely fused larva of Driesch. There is, on the contrary, definite evidence that disintegration of skeletal materials takes place in the suppressed larva *without any corresponding enlargement of the skeleton or the body of the dominant larva.* The dominant larva in figures 12, 13, 14, and 15 are no larger than in figures 9, 10B, 5, 6, 7B, and 8—all drawn to the same scale—or figures 11, 10B, and 7A, drawn to greater magnification. In the second place the dominant larva in these fusions are no larger than the corresponding control larva, and the variation in size is approximately the same in both groups. There are no giant larva in the sense employed by Driesch and Morgan and other investigators. There are completely fused larva as there are completely fused blastulæ and gastrulæ.

The facts clearly show that in the blastula, gastrula, and larval stages the less or earlier differentiated tissues (such as ectoderm and entoderm) after their union to form the giant body and giant gut diminish in size until a normal single body and gut are approximated. This diminution must occur either as a consequence of the degeneration of certain cells or the diminution in size of the cells. The latter can readily be shown not to be the case.

It can also be shown that the degenerating substances in one larva do not serve as food or stimulant of the other, for the dominant larva are no larger nor do they develop any faster than the controls. In fact they are considerably slower than the controls.

The increased growth occurs not in any increase of the larval body or of the contained organs, but in the hypertrophied and supernumerary processes and bars whose origin and development have been described.

SUMMARY.

A new method was discovered by which large numbers of eggs of *Toxopneustes variegatus* could be fused together, during the blastula and gastrula stages. The method consists essentially in placing the fertilized eggs in a solution composed of one part of sea-water to three parts of either an isotonic or a slightly hypotonic NaCl solution.

This solution has two effects upon the eggs, namely, it increases the volume of the egg and thereby bursts the fertilization membrane, and it gelatinizes the surface of the egg. When any such eggs are in contact they become agglutinated and may remain agglutinated and produce twin larvæ or fuse together during the blastula or gastrula stages.

The conditions that favored the fusion process were:

(1) The number of agglutinated eggs: More than two eggs rarely gave rise to a fused and characteristic larvæ. When two eggs were agglutinated they either developed into twin larvæ or fused more or less completely together..

(2) Time of fusion: The earlier in development that fusion occurred the more completely did the parts tend to fuse together and vice versa.

(3) Area of common surface: The greater the surface common to both eggs or blastulæ, the more completely and the earlier did they tend to fuse, and vice versa.

(4) Differential rate of development: When the two embryos developed equally fast, each was essentially perfect, though fused to the other. When one developed less rapidly than the other, reciprocal changes took place, as a result of which the larvæ tended toward complete fusion and form equilibrium.

Form equilibrium is determined by at least these three factors, namely:

(1) Differential rate in the development of the two eggs.

(2) An inhibition in the development of certain structures. The skeleton in the more slowly developing larva was rarely complete, lacking those portions of the skeleton that were last differentiated. Such portions may include one-half, three-quarters, or more of the skeleton. The parts that do appear are perfectly normal.

(3) A disintegration of certain structures. Skeletal parts of the larva after their complete differentiation may break down and completely disappear. Such decrease in the skeleton was accompanied by a decrease in the corresponding parts of the body of the larva.

Such regulative changes are nearly always limited to the more slowly developing larva. They result in the complete atrophy of this larva without any reciprocal or compensatory effect upon the shape, size, or parts of the more rapidly developing or dominant larva, except for the skeleton.

The ectoderm and the entoderm of one larva may fuse with that of the other, and may subsequently be absorbed or be disintegrated without any visible effect upon the other larva. The skeleton never fused. When it was suppressed in one larva, some or all of the unused mesenchyme cells migrated to other regions of the same larva, or into the adjoining larva, and there gave rise to hypertrophied or supernumerary parts of the skeleton, and in certain instances replaced in part the missing skeleton of the suppressed larva.

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VII.

EXPERIMENTS ON THE PERMEABILITY OF CELLS.

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Three text-figures.

EXPERIMENTS ON THE PERMEABILITY OF CELLS.

By J. F. McClendon.

One of the most important steps in the analysis of life was the discovery of oxygen. Ever since that time it has been known that animals absorb free oxygen and give it out in a combined form.

The discovery of enzymes has given a clue to the rapidity of oxidation within cells; but enzymes are always present in living substance, and yet oxidation may vary from almost zero in certain eggs and seeds during rest to a high rate during activity. We know that oxidation within muscle is greatly increased during contraction, yet hardly anyone would suppose this to be due to the sudden increase of oxidases. Loeb, however, did use this explanation for the increased oxidation of the egg on fertilization, by suggesting that the sperm carries an oxidase into the egg. Certainly the sperm contains an oxidase, as does every other cell, but the sperm is minute in comparison with the egg, and no one has found any indication of an unusual amount of oxidase in the sperm. Furthermore, the egg may be made to divide by artificial means without the introduction of any enzyme.

An organism may be caused to absorb more oxygen by any one of a number of different stimuli. But what is stimulation? What is the first change induced in the organism or excised organ by all stimuli?

Pfeffer showed that stimulation of sensitive plants causes a change in permeability of certain cells (of the pulvinus). This change in permeability causes a change in turgor (internal osmotic pressure) and movement results. The question arises: what relation does this bear to oxidation? According to Ralph Lillie oxidation is suppressed in the cell by the accumulation of waste products to which the resting cell is impermeable. Stimulation increases the permeability of the cell to them and consequently oxidation is increased. Lillie maintained that on fertilization the egg became more permeable, and linked all subsequent phenomena with this change. Not finding any other explanation of the stimulating action of the sperm, I attempted an exact study of the permeability of the egg.

EXPERIMENTAL.

Three methods of procedure were followed: (1) the use of cell masses as partitions (on eggs of *Lytechinus*); (2) the use of quantities of eggs suspended in a liquid medium (on eggs of *Fundulus*); (3) experiments on individual eggs (of *Arbacia*).

The first of these methods was tried at the Tortugas laboratory on the eggs of the sea-urchin *Lytechinus* (*Toxopneustes*) *variegatus*. Advantage was taken of the fact that the electrolytes of the eggs and the medium dissociate into ions which bear electric charges, and therefore their movement through the eggs could be detected, with a high degree of accuracy, by the electric conductivity method of Kohlrausch.[1]

Several bushels of sea-urchins were collected each day and the eggs of the ripe females placed in large dishes of sea-water. The mucus egg membranes (jelly, zona pellucida) were washed off and the eggs were introduced into a conductivity vessel made especially for the purpose (fig. 1). The conductivity vessel was placed in the centrifuge and the eggs were precipitated until they were closely packed together. By microscopic examination it was found that only a trace of sea-water was left in the spaces between the eggs (fig. 2).

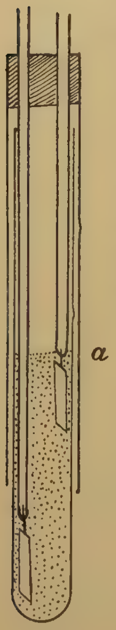

FIG. 1.

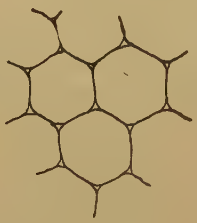

FIG. 2.

By raising the electrodes above the level of the eggs they came to lie in the supernatant sea-water that had been pressed out from between the eggs. In this way the conductivity of sea-water and egg-mass could be measured separately within two minutes. The conductivity of the egg-mass, when moderately centrifuged, was about one-twentieth of that of the sea-water, indicating that the conductivity of the egg itself must be almost nil.

The conductivity of the egg-mass was greatly affected by the degree of packing. However, the vessel was so long and narrow that the packing of the eggs could be recorded accurately by marking their upper limit on the side of the vessel (fig. 1, *a*). The eggs could then be removed from the conductivity vessel, replaced, and centrifuged to the same level, when the conductivity was found to be the same. In this way I was able to measure the conductivity before and after fertilization and before and after artificial stimulation causing cleavage. It was found that the conductivity increased about one-fourth when the developmental processes began. The sources of error and methods of controlling them are discussed in another paper.[2]

One point, however, the elimination of the fertilization membrane, needs further consideration. Soon after the egg is fertilized, a fine line appears close around it and gradually expands until it forms a circle, widely separated from the egg. Almost every one (except Kite) supposes this to represent a spherical membrane, the "fertilization membrane." Before fertilization the egg is covered by a thick transparent jelly or mucus layer, called the chorion or zona pellucida by some authors. I stained this jelly with basic dyes (neutral red and methylene blue). These dyes coagulate the jelly, causing it to become membranous.

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[1] Ostwald-Luther. Messungen.

[2] McClendon. Amer. Jour. Physiol., vol. 27, p. 240.

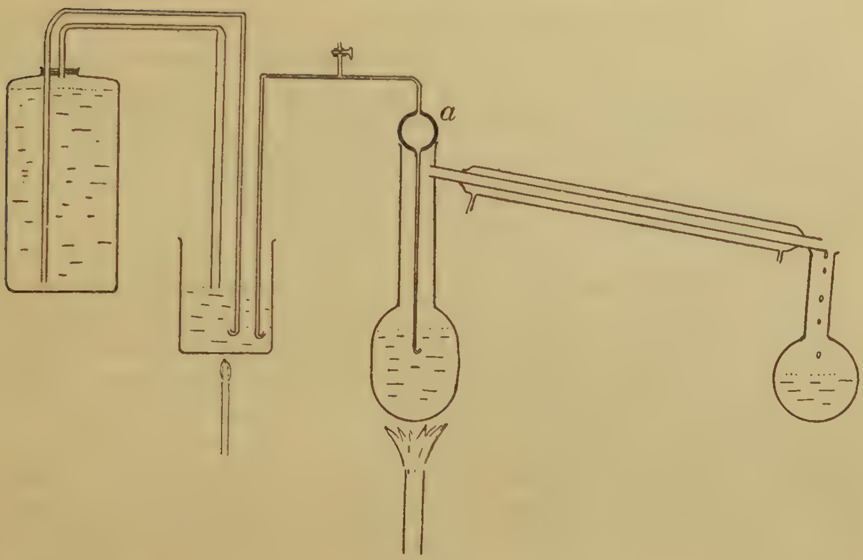

FIG. 3.

In these experiments all toxic substances were eliminated from the water by redistilling it in the apparatus shown in figure 3. The stock bottle (to the left) is filled with distilled water, which siphons over into the beaker and keeps the latter filled up to the mouth of the wide tube. A small flame under the beaker boils the water and drives out all dissolved gases (since the water contains no salts to hold back CO_2, and no non-volatile substances to liberate ammonia). The boiling water from the beaker siphons over into the Kjeldahl flask over a large flame. If the siphon be drawn out into a fairly small opening the Kjeldahl flask may be set lower than the beaker, as in the figure; but this has the disadvantage that when the still is cooled the Kjeldahl flask fills up to the neck and makes it inconvenient to start again. The siphon has a bulb blown on it at *a*, to stop the mouth of the flask. It is not necessary that this stopper be ground in, provided it fits fairly snug, as water condenses on the stopper and seals the crack. A hole is blown in the side of the neck of the flask to admit the condensing tube. This hole may be reamed out with the end of the tube itself, while the glass is hot, to insure a fairly close fit. The condensing tube is of fused quartz and the only water that collects in the receiving bottle is condensed on the quartz. The cold water dissolves glass very slowly, so that it is permissible to use a glass receiving bottle if the water is distilled fresh each day. Hard glass (Jena or Bohemian) dissolves more slowly but contains heavy metals which are toxic even in the minute quantities that dissolve away in the cold distilled water, even though the glass be previously steamed out. Soft glass liberates traces of sodium, calcium, and potassium, which render the water less toxic, but which, if present in sufficient quantity, may be a source of error in the experiment.

In a previous paper[1] I concluded that the fertilization membrane is the result of a mutual coagulation and precipitation between the jelly and another transparent colloid filling the space between it and the egg. I found that if the jelly be removed from the egg no fertilization membrane can be formed. Elder[2] subsequently made the same observation and came to the same conclusion. No matter what is the nature of the membrane, the fact that it can be suppressed is of importance in my technique, since the presence of the membrane might be considered as a source of error.

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[1] McClendon. Science, vol. 33, p. 387, Mar. 10, 1911. [2] Arch. f. Entwicklungmech., vol. 33, p. 143, 1912.

The increased conductivity of the eggs indicates increased permeability to electrically charged particles, since electrons can not pass in the free state through solutions without very soon attaching themselves to atoms. The particles concerned must be ions, since colloidal particles move slowly and carry little electricity per unit mass, and could hardly cause the great increase in conductivity observed.

This increase in permeability was confirmed by plasmolytic experiments on the eggs of *Arbacia* at Woods Hole.

What ions are concerned in the increase in permeability has not been determined. Experiments which I made on the *Fundulus* egg are suggestive.[1] *Fundulus* eggs (which normally develop in sea-water), when developing in distilled water, do not give up chlorides to the water. If placed in sulphates or nitrates of sea-water metals, they still do not give up chlorides. If placed in distilled water they do not give up magnesium (or calcium). But if placed in sodium salts they lose magnesium (and probably calcium and potassium), and chlorine, because the pure sodium increases the permeability.

A critical review of the literature on permeability is given in a previous paper[2] but I shall here refer to some of it briefly. In the summer of 1910 R. Lillie, Lyon and Shackell, E. N. Harvey, and the author, all working on the sea-urchin's egg, had come to the conclusion that its permeability increased on fertilization. Very shortly there appeared a paper by Loeb[3] in which he made this statement:

> In former papers, especially in a book published a year ago, I pointed out that the process of membrane formation, or a certain alteration of the surface of the egg, is the essential cause of the development of the egg; and I pointed out, also, that this alteration of the surface might increase the permeability of the egg, especially for hydroxyl ions.

I have found one such paper,[4] but it was directed against the view that the cell is impermeable to electrolytes, and gave evidence to show that the egg is more permeable to salts than to sugar. In the book referred to[5] Chapter XIII deals largely with permeability and ends with the following conclusion (on page 118):

> Wir kommen also zu dem Schluss, dass die Hydroxyloinen des Seewassers auch in das unbefruchtete Ei diffundieren, und dass die Membranbildung des Oxydationen im Ei nicht dadurch steigert, dass sie die Durchgängigkeit desselben für Hydroxylionen erhöht, sondern dass sie die für die Entwicklung nötigen Oxidationen auf anderem Wege entfesselt oder möglich macht.

If Loeb has found evidence that the permeability of the egg increases on fertilization, or fatty acid treatment, I would be very glad to have my attention called to it, but it is difficult to find a paper without a reference to the journal in which it is published.

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[1] Amer. Jour. Physiol., vol. 29, p. 295, Appendix II.
[2] McClendon. Biol. Bull., vol. 22, p. 113. 1912.
[3] Loeb, J. Science, n. s., vol. 32, p. 412. Sept. 23, 1910.
[4] Loeb. Univ. of California Publications, Physiol., vol. 3, No. 11, p. 81. 1908.
[5] Loeb. Die Chemische Entwicklungserregung des tierischen Eies. Berlin. 1909.

The degree of permeability is of importance in development. R. Lillie maintains that too great an increase in permeability causes cytolysis of the egg. Gray[1] attempts to explain the abnormality of Echinoderm hybrids by assuming that the foreign sperm produces the wrong degree of permeability and this leads to some of the chromatin becoming pathological and being thrown out of the spindle.

The changes in permeability are probably due to changes in the aggregation state of colloids: either proteids, lipoids, or proteid-lipoid combinations or associations. Many investigators have looked for such changes with the microscope. That some change takes place at the surface of the egg on fertilization has long been known, but opinions differ as to its exact nature. Spherical concentric surfaces, when viewed tangentially with the microscope, cause optical illusions, and the real structure can not be exactly determined without knowledge of the refractive indices of the substances composing it. Kite, however, claims that a thin layer may be observed on the surface of the unfertilized egg and, on fertilization, this layer absorbs water and swells. He claims to have stained it differentially.[2] I do not understand, however, his observations on unstained eggs. He says that this layer which he calls the vitelline membrane becomes more distinct (of different refractive index) from the sea-water on swelling, whereas it is well known that all hydrophile colloids, on swelling, become less distinct and more nearly of the same refractive index as the medium.

The swelling of this layer might cause an increase in permeability, since the swelling of all hydrophile colloids increases their permeability to water-soluble substances. Lillie, however, believes that a reversible increase in permeability takes place in the superficial layer of the cytoplasm (plasma membrane) and J. Gray who has confirmed my determination of the increase in permeability of the egg finds that impermeability returns in 15 minutes, whereas Kite's vitelline membrane remains swollen. I consider Kite's vitelline membrane a new name for a colloid postulated by Loeb.

Mitchell and the author[3] studied experimentally the role of hydroxyl ions in the increase in oxidation of sea-urchin's eggs, which follows fertilization or artificial stimulation. We concluded that the increased permeability allows the hydroxyl ions of the sea-water to penetrate the egg. This increase in hydroxyl ions within the egg might increase oxidation.

CONCLUSIONS.

The permeability of the egg to ions and perhaps some other substances increases on fertilization. The unfertilized egg is perhaps in a dormant condition and the increase in permeability probably allows a rapid interchange with the surrounding medium necessary for activity (development).

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[1] Gray, J. Proceedings of the Cambridge Philosophical Society, vol. 17, p. 1, 1913.
[2] Kite. Science, n. s., vol. 36, p. 562. 1912.
[3] Jour. Biol. Chem., vol. 10, p. 459.

Whereas this supposed significance of permeability has not been proven, the sea-urchin's egg is not an exception. Spores, eggs, seeds, and pupæ are usually inclosed in relatively impermeable membranes. A striking example is the cocoa nut. Whereas an active organism may be partly inclosed in a relatively impermeable skin, great activity is always associated with a rapid interchange with the medium, and this is possible through more permeable portions (lungs, gills, kidneys, gut).

The relation of permeability to oxidation can hardly be determined until more is known about the mechanism of animal oxidations. These seem to depend on structure since complete oxidations cease when structure is completely destroyed. Reference is made only to oxidations resulting in the formation of $CO._2$ Oxidizing enzymes such as tyrosinase, which are independent of structure, do not completely oxidize the substances acted on.

VIII.

THE RELATION BETWEEN THE RATE OF PENETRATION OF MARINE TISSUES BY ALKALI AND THE CHANGE IN FUNCTIONAL ACTIVITY INDUCED BY THE ALKALI.

BY E. NEWTON HARVEY,
Instructor in Physiology, Princeton University.

One figure.

THE RELATION BETWEEN THE RATE OF PENETRATION OF MARINE TISSUES BY ALKALI AND THE CHANGE IN FUNCTIONAL ACTIVITY INDUCED BY THE ALKALI.

By E. Newton Harvey.

OBJECT AND METHOD.

The present study, made at Tortugas in the summer of 1911, is a continuation of permeability investigations undertaken at Columbia University in 1910 to 1911.[1] My aim has been twofold. First, to compare the permeability of the cells and tissues of salt-water organisms with those of fresh-water forms. Second, to determine the relation between the rate of penetration of the alkali and the appearance of structural or functional changes in the cell. Rate of penetration of the alkali may be best determined by the color change of some indicator within the cell. As in previous work, neutral red was used for the purpose. It is non-toxic, readily staining the living cell, and the color change (of red in acid or neutral to yellow in alkaline solution) is sharp and well marked.

Neutral red was first made use of as an indicator in studying permeability by Bethe.[2] Medusæ stained in the dye become a deep orange-red, which changes to bright red in HCl and to yellow in NaOH.

Warburg[3] found that sea-urchin eggs stained in neutral red were entered readily by NH_4OH but not by NaOH. My own experiments[4] gave similar results for another species of sea-urchin (*Toxopneustes variegatus*) and for *Paramœcium*, *Elodea*, and *Spirogyra* cells.

The penetration of the following alkalies was studied:

1. *Weakly Dissociated:*
   Ammonia—NH_4OH
   Methylamine—NH_3CH_3OH
   Dimethylamine—$NH_2(CH_3)_2OH$
   Trimethylamine—$NH(CH_3)_3OH$
   Ethylamine—$NH_3C_2H_5OH$
   Normal propylamine—$NH_3C_2H_5CH_2OH$
   Isopropylamine—$NH_3(CH_3)_2CHOH$

2. *Strongly Dissociated:*
   Tetraethylammonium hydroxide—$N(C_2H_5)_4OH$
   Sodium hydroxide—NaOH
   Potassium hydroxide—KOH
   Strontium hydroxide—$Sr(OH)_2$

The addition of the stronger alkalies to sea-water precipitates the Mg so that a Mg-free sea-water containing 0.625 m. (100 NaCl + 2.2 KCl + 2.5 $CaCl_2$) was used to dissolve the alkali. Such a solution is a fairly well-balanced medium for marine tissues, being only slightly inferior to van't Hoff's solution. The salt present in solution with the alkali affects also the rate of entrance of the latter, so that it is important to use always the same type of salt solution for comparative results. As stated above, Mg-free sea-water of 0.6 m. concentration was employed.

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[1] Harvey, E. N. Jour. Exp. Zool., vol. 10, p. 507. 1911.
[2] Bethe. Pfluger's Archiv, vol. 127, p. 219. 1909.
[3] Warburg, O. Zeit. f. Physiol. Chem., vol. 66, p. 305. 1910.
[4] Harvey, E. N. Loc. cit.

The magnitude of the difference between pure NaCl and a balanced medium is brought out in table 1.

TABLE 1.

Egg.	Concentration of NaOH.	Color change in Mg-free sea-water.	Color change in 0.6 m. NaCl.
		min.	min.
Toxopneustes variegatus	N/100	10 to 12	5
Palolo-worm (Eunice fucata)	N/80	12	5
Holothuria floridana (immature)	N/80	6	1

PART I. THE PERMEABILITY OF MARINE TISSUES.

PENETRATION RATE AND DEGREE OF DISSOCIATION.

Just as in the case of fresh-water organisms (*Paramœcium*, *Spirogyra*, *Elodea*), the weakly-dissociated alkalies (group 1) penetrate marine tissues almost instantly, the strongly dissociated only after an interval of 30 to 60 minutes. This statement applies to all the cells or tissues of salt-water organisms investigated, including the following:

Holothuria floridana, immature eggs.
Holothuria floridana, respiratory tree.
Hipponoë esculenta, mature eggs.
Toxopneustes variegatus, mature eggs.
Toxopneustes variegatus, plutei.
Sabella sp., tentacles.
Eunice fucata (palolo-worm), segmenting eggs.
Eunice fucata (palolo-worm), trochophores.
Pomatostegus stellatus, mature eggs.
Cassiopea xamachana, gonads.
Cassiopea xamachana, subumbrella epithelium.
Two unidentified species of marine algæ.

Thus, the anomalous result again appears—that it is the least dissociated, the weakest group of alkalies, which penetrate living cells most rapidly. Two of the members of this group are considerably less dissociated than the rest—ammonia and trimethylamine. We might expect them to penetrate most rapidly of all if rate of penetration bears an inverse relation to degree of dissociation. There is, however, no marked difference in penetration rate between ammonia or trimethylamine and the other amines,[5] but a well-defined difference in toxicity.

TOXICITY AND DEGREE OF DISSOCIATION.

The lower toxicity of ammonia and trimethylamine is indicated in table 2. Efficiency in preventing development of the sea-urchin's egg and cytolytic action were taken as criteria of toxicity. In addition to ammonia and the amines, tetraethylammonium hydroxide was also studied. Although a substituted ammonia, it is strongly dissociated, ranking with the inorganic alkalies, and in table 2 it may thus serve as a representative of the inorganic alkalies. The unfertilized eggs were placed in the solution, and a certain number removed to sea-water after intervals of 2 and 5 minutes and fertilized.

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[5] Ammonia and the amines enter cells so rapidly that it is impossible to detect constant differences in penetration rate.

TABLE 2.—*Effect of ammonia, amines, and tetraethylammonium hydroxide on toxopneustes eggs, stained in neutral red.*

Solution of alkali in Mg-free sea-water N/250-concentration.	Color change of red to yellow.	Effect of solution on egg after 3 hours.	Condition of eggs removed to sea-water after 2 minutes and fertilized. Examined after 3 hours.	Condition of eggs removed to sea-water after 5 minutes and fertilized. Examined after 3 hours.
NH_4OH	Instantly	Irregular fragmentation and globule formation.	Segmentation; some irregular.	Many irregular segmentations.
Methylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs; a few fragments.
Dimethylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs; a few fragments.
Trimethylamine	Instantly	Irregular fragmentation and globule formation.	Segmentation; some irregular.	Many irregular segmentations.
Ethylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs and fragments.
Propylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs and fragments.
Isopropylamine	Instantly	Clear cytolysis	Irregular segmentation.	Unsegmented eggs; some irregular segmentations.
Tetraethylammonium hydroxide.	Not yellow after 3 hours.	Many red eggs, and some fragments.	Irregular segmentation; red in color.	Irregular segmentations; red in color.

I. Control—fertilized in Mg-free sea-water—red, segmentations normal.
II. Control—unfertilized in Mg-free sea-water—red, unsegmented, normal.

It will be noticed that eggs are not cytolyzed after 3 hours immersion in N/250 NH_4OH, $NH(CH_3)_3OH$, and $N(C_2H_5)_4OH$ (column 3) and after 5 minutes immersion are still capable of fertilization (column 5); whereas the opposite is true of the remaining alkalies of table 2. $N(C_2H_5)_4OH$, most highly dissociated of all, is no more toxic than NH_4OH, least dissociated of all. The explanation of the difference must lie in the penetrating powers of the two alkalies. $N(C_2H_5)_4OH$ can not penetrate the egg-surface and is consequently less toxic than NH_4OH, which readily penetrates. Penetration is of first importance in determining toxicity. If the alkali penetrates readily, then degree of dissociation is a further factor in determining toxicity. Those weak (ammonia and trimethylamine) alkalies which are least dissociated are least toxic.

The same fact appears when other cells are tested; for if segmenting palolo eggs (*Eunice fucata*) are placed in N/250 solutions of NH_4OH and the amines for two minutes, and then returned to sea-water, only those eggs develop into swimming larvæ which have been immersed in trimethylamine and ammonia. Eggs exposed to the remaining amines are for the most part killed; only a few larvæ develop.

GENERAL CONSIDERATIONS.

On being returned to sea-water the palolo eggs which have been turned yellow by immersion in the weak alkalies again become red in color. The return of color is much more rapid in ammonia and trimethylamine than in the remaining amines. The explanation of this phenomenon lies in the lesser degree of dissociation of the former substances. Neutral red occurs in the egg in small granules, a combination between the neutral red base (BOH) and some organic substance having acid properties (HA), forming a salt (BA).

$$BOH + HA = BA + H_2O$$

This red salt BA is broken up by NH_4OH (or any other alkali), neutral red (BOH) is freed, and a compound NH_4A is formed. The weaker the alkali the greater will be the hydrolytic splitting which its salt, NH_4A, will undergo.

$$NH_4A + H_2O = NH_4OH + HA$$

The NH_4OH will diffuse away more rapidly than a stronger base, such as methylamine, and recombination of HA and BOH will consequently occur more rapidly with the reappearance of the red color.

The results with marine organisms agree in general with those obtained on fresh-water forms. One point of difference is worthy of mention. Ammonia and trimethylamine were found to be less toxic than the remaining amines for *Elodea* cells, just as in the case of marine forms; but while ammonia was less toxic, trimethylamine was ranked with the remaining amines, not with ammonia, for *Paramœcium*. I am unable to explain this difference. It is possible that my result with trimethylamine and *Paramœcium* may be due to some unforeseen experimental error. In other respects the results agree entirely.

The effect of alkalies on living tissues in general may be represented as follows:

Strongly dissociated inorganic alkalies, including tetraethylammonium hydrate.	More strongly dissociated of the weak alkalies. Amines (except trimethylamine).	Less strongly dissociated weak alkalies. Ammonia and trimethylamine.
Penetration very slow. Least toxic.	Penetration very rapid. Most toxic.	Penetration very rapid. Less toxic.

PART II. PERMEABILITY AND FUNCTIONAL CHANGE.

THEORETICAL.

Two quite opposite points of view have been taken by physiologists in regard to the relative importance of the permeability of cells. Some regard the cell-surface as of no consequence in regulating cell processes, being freely permeable at all times to crystalloidal substances in the medium or in the cell. Others see in the surface an important regulator of chemical reactions in the cell and a barrier of a high degree of impermeability, retaining cell constituents as a test-tube retains the substances reacting within its walls. Theoretically, no more simple method of regulating the velocity of chemical reactions could be found than by means of a membrane whose permeability to the reaction product might vary. Actually, there is a large mass of evidence indicating that the cell-surface is a highly impermeable membrane for substances normally occurring in or used by cells. The evidence has been advanced in numerous papers by Lillie[6] and Hoeber[7] and need not be discussed here.

Crucial evidence has only recently been put forward that the cell-surface (particularly its permeability) plays an important part in cell activities—that it may vary at different periods of activity and in the presence of definite substances.

The evidence may be classified under three heads:

1. Measurements of permeability changes induced by narcotics, anæsthetics, and salts or other substances which affect cell activities.
2. Measurements of permeability during different states of functional activity.
3. Determinations of the rate of penetration of a substance and rate of change in functional activity induced by the substance.

PERMEABILITY CHANGE THROUGH CHEMICALS.

Of particular interest here is the experiment of Osterhaut,[8] in which it is shown most clearly by the plasmolytic method that the plasma membrane possesses greatest impermeability to salts in a balanced medium or in the medium which most nearly approaches that of the normal. Pure NaCl or pure $CaCl_2$ both enter *Spirogyra* cells or marine algæ slowly, the latter more slowly than the former; but if the two are mixed in the proportions of a balanced solution, they mutually retard each other's entrance. The NaCl, alone in solution, must increase the permeability of the plasma membrane to such an extent that its entrance is greatly facilitated.

Osterhaut[9] has recently confirmed his results, mentioned above, by the electrical conductivity method of measuring permeability. Disks cut from the kelp, *Laminaria*, were placed face to face so as to form a cylinder several centimeters long, and its conductivity was measured. In NaCl the conductivity of the cylinder was found to increase regularly (as compared with its value in sea-water), while in $CaCl_2$ or lanthanum chloride the conductivity decreased. Under the conditions of the experiment, increase or decrease in conductivity denotes a corresponding increase or decrease in permeability. The changes in permeability induced by NaCl, $CaCl_2$, or $LaCl_3$ are readily reversible within certain limits on returning the tissue to sea-water.

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[6] Lillie, R. S. Amer. Jour. Physiol., vol. 24, p. 14. 1909; vol. 28, p. 197. 1911. Also Biol. Bull., 27, 192. 1909.

[7] Hoeber, R. Physikalische Chemie der Zelle und Gewebe., 3d Aufl. 1911.

[8] Osterhaut, W. J. V. Science, n. s., vol. 34, p. 187. 1911.

[9] Osterhaut, W. J. V. Science, n. s., vol. 35, p. 112. 1912.

My own experiments, cited above, indicate that NaOH may enter many kinds of eggs much more readily when dissolved in 0.6 m. NaCl than in 0.625 m. Mg-free sea-water. The pure NaCl evidently decreases the resistance[10] of the egg-surface for NaOH.

The surface of *Elodea* and *Spirogyra* cells is likewise affected by minute traces of substances in solution. For instance, N/40 NaOH dissolved in tap-water entered *Spirogyra* in about 40 minutes; dissolved in double distilled water, condensed in soft glass,[11] in about 10 minutes; and dissolved in N/10 NaCl, instantly. Again the resistance of the surface is greatest in the medium most nearly normal. Addition of $CaCl_2$ to the NaCl prevents so rapid penetration of NaOH.[12]

Anæsthetics give similar results with *Elodea* cells. Chloroform added to N/40 NaOH to 1/6 saturation increases the penetration rate from 90 to 13 minutes. The protoplasmic rotation characteristic of the *Elodea* cells ceases in 1/6 saturated chloroform, but the effects of the chloroform are completely reversible, for, on removing the leaves again to pure water, rotation promptly begins. Alcohol and ether act in a similar manner.

That chloroform increases permeability in 1/6 saturated solution may be verified by the plasmolytic method. Such "reversibly" chloroformed cells are plasmolyzed by urea much less readily than normal cells, a result indicating that the urea enters more rapidly.[13]

On the other hand, Lillie[14] cites evidence to show that very small concentrations of anæsthetics decrease permeability.

PERMEABILITY AND FUNCTIONAL ACTIVITY.

Lillie[15] has advanced evidence showing that stimulation of muscle-tissue is accompanied by an increase in permeability of the tissue. Those sodium salts which are most effective in stimulation are most effective in causing the loss of pigment from the tissues of *Arenicola* larvæ. Loss of pigment indicates increased permeability to pigment. The same is true as regards artificial parthenogenesis. These salts most effective in stimulating development cause most rapid loss of pigment from the eggs (*Arbacia*);[16] they are also most toxic; in them death of the egg ensues most rapidly. It has long been known that the condition of death is one characterized by marked increase in permeability. The change on stimulation is in the direction of that accompanying death.

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[10] The word resistance is used instead of permeability to indicate that the cell is normally impermeable to NaOH which only enters after affecting the cell-surface.

[11] Such water is perfectly harmless for both *Spirogyra* and *Paramœcium*, a good test of the purity of a water.

[12] Harvey, E. N. Loc. cit., p. 540.

[13] Harvey, E. N. Loc. cit., p. 539.

[14] Lillie, R. S. Amer. Jour. Physiol., vol. 29, p. 372, 1912; vol. 30, p. 1, 1912.

[15] Lillie, R. S. Amer. Jour. Physiol., vol. 24, p. 14. 1909.

[16] Lillie, R. S. Amer. Jour. Physiol., vol. 26, p. 106. 1910.

Further evidence that the egg increases in permeability immediately after fertilization is furnished by McClendon.[17] He found that the electrical conductivity of the sea-urchin egg is increased after fertilization, thus showing that its permeability is increased.

A similar result may be obtained by the plasmolytic method.[17] Fertilized eggs are plasmolyzed more readily than unfertilized by a molecular solution of cane sugar. This is due to the fact that the salts diffuse out of fertilized eggs more rapidly, the osmotic pressure within falls more rapidly, and plasmolysis occurs more readily.

Yet another fact indicates that the surface of fertilized eggs has undergone a marked change. They are entered more readily by NaOH.[18] In this particular case we may say that the resistance to the entrance of alkali has decreased, using the word resistance, as before, to indicate that the NaOH enters only after modifying the normal impermeability of the surface.

Tröndle,[19] and also Lepeschkin,[20] using the plasmolytic method, have found that the permeability of plant cells for salts and sugar increases in the light. The increased permeability has an adaptive significance in that the sugar formed by photosynthesis may be more rapidly removed and photosynthesis itself thereby favored.

PENETRATION RATE AND CHANGES IN FUNCTIONAL ACTIVITY.

Under this head are classed the cases in which the rate of penetration of the substance is reduced to zero, *i. e.*, cases in which marked functional or structural changes appear, while we have no evidence that the substance enters the cell at all. The obvious conclusion is that it produces the effects observed by acting on or combining with the cell-surface itself, and affords excellent proof of the important rôle played by the surface in all activities.

It has long been known that muscles lose their irritability in isotonic solutions of potassium salts. Overton[21] showed that the muscle might remain in the potassium salt solution for days without change of volume, so that the potassium salt could not have entered and its effect in changing irritability must have been a surface action.

Warburg[22] showed that the oxygen consumption of the egg was greatly increased in the presence of dilute NaOH, although the NaOH did not enter; yet NH_4OH could enter readily and in concentrations containing too few OH ions to affect to any marked extent the oxygen consumption. Neutral red is therefore a more delicate test for OH ions than increased rate of oxidation. Here is unequivocal evidence that a chemical process in a cell can be affected by a substance which does not enter the cell at all.

In my earlier paper I have shown that initiation of cell division in sea-urchin eggs, stoppage of ciliary movement and cytolysis of *Paramœcium*, and stoppage of protoplasmic rotation in *Elodea* might be brought about before any appreciable amount of NaOH had entered the cells.

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[17] McClendon, J. F. Amer. Jour. Physiol., vol. 27, p. 240. 1910

[18] Harvey, E. N. Science, n. s., vol. 32, p. 565. 1910.

[19] Tröndle. Ber. d. deutsch. bot. Ges., vol. 27, p. 71, 1909; and Jahrb. f. wiss. Bot., vol. 48, p. 171, 1910.

[20] Lepeschkin. Ber. d. deutsch. bot. Ges., vol. 26*a*, pp. 198, 231, and 724, 1908; and Beihefte z. bot. Zentralb., vol. 24I, p. 308, 1910.

[21] Overton, E. Pfluger's Arch., vol. 92, p. 115. 1902.

[22] Warburg. Loc. cit.

It is the purpose of the following paragraphs to indicate how far other functions may be affected by NaOH before it penetrates the cell. It may be stated in advance that every cell activity thus far studied is affected, indeed abolished, before the NaOH enters, while a great many cell activities remain entirely unaffected even after NH_4OH has penetrated and turned the neutral red to yellow.

Evidence showing that the indicator method is an adequate one for detecting the penetration of both strong and weak alkalies has been presented in a previous paper (Amer. Jour. Physiology, vol. 31, 1913).

EXPERIMENTAL.

MUSCLE AND NERVE TISSUE.

CASSIOPEA XAMACHANA.

The only experiments on the permeability of the cells of medusæ of which I am aware are those of Bethe, cited above.

The method of experimentation with *Cassiopea*, a scyphomedusa well adapted for physiological investigation, was the same as that previously used in studying the effect of temperature on the muscular contraction and nerve conduction.[23] The method was originally described by Mayer.[24] A long strip of tissue cut from the disk of the jelly-fish is laid across three dishes (A, B, and C, fig. 1). Dishes A and C are filled with sea-water. Dish B is filled with the alkali solution to be tested. Stimuli are constantly arising in the sense-organs in dish A and pass along the nerve-network of the strip of tissue toward C, stimulating the muscles as they go. When the nerves are so affected by the alkali in B that they no longer conduct a stimulus, the muscle in C will no longer contract. In the case of some alkalies, acids, and most other substances, the *muscles* in B are affected before the nerves. The time required to stop contraction can be determined by mere inspection, since the muscles are being constantly stimulated by nervous impulses carried from the sense-organs in A.

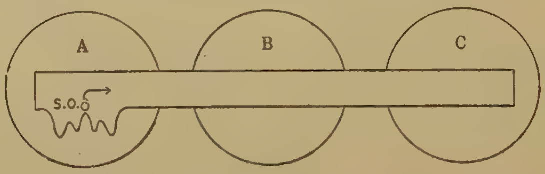

FIG. 1.

In the case of certain of the alkalies, the *nerves* in B are affected before the muscles. We may then determine by direct stimulation of the muscles in B with the electric current when they have been so far affected as to cease contracting.

Entrance of alkali is of course detected by the color change of neutral red.

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[23] Harvey, E. N. Carnegie Institution of Washington, Pub. No. 132, p. 27. 1911.
[24] Mayer, A. G. Carnegie Institution of Washington, Pub. No. 102, p. 128. 1908.

The subumbrella surface of *Cassiopea* is covered by a thin layer of epithelium and muscle and nerve tissues. The epithelium is outermost, nerve-network next, then come the muscle-fibers, and finally, embedded in the jelly, are clumps of symbiotic algal cells scattered in lines running parallel with the muscle-fibers.

Microscopic examination of the *Cassiopea* strips used in the following experiments reveals a layer of red-stained granules near the surface and on focusing downward *lines* of red-stained granules are observed following the muscle-fibers and appearing to be within them. Transverse sections of the same strips showed, however, that very little more than the outer third of the epithelio-muscle layer contained cells stained in neutral red. The dye is chiefly taken up by globules and granules in epithelial mucus-cells. These granules are practically all cast off in the mucus formed by exposing *Cassiopea* to chloroform-saturated sea-water or other injurious solutions. Neither nematocysts nor algal cells stain. No definitely stained nerve-cells can be made out, although red-stained granules are present where nerve-cells should be. Only the region of the muscle-fibers away (*i. e.*, toward epithelium) from the contractile fibrils stain. It should be borne in mind, then, in interpreting the following results, that red-stained granules are not present in the immediate region occupied by the contractile fibrils, but are present in a region first reached by alkali diffusing into the tissues and in a region containing the nerve-network, although it is impossible to say whether granules within the nerve-cells are stained or not. It is probable, however, that both nerve-cells and the outer ends of the muscle-fibers contain the red indicator. The results are given in the following table:

TABLE 3.—*Relation between color-change, cessation of contraction, and cessation of conduction in Cassiopea. (Tortugas, July 9–10, 1911.)*

Alkaline solution N/250 concentration in Mg-free sea-water.	Time of exposure to solution in minutes.	Color change in minutes.	Cessation of contraction in minutes.	Cessation of conduction in minutes.	Recovery of contraction in minutes on removal to sea-water.	Recovery of conduction in minutes on removal to sea-water
Ammonia	20	1.5	Feeble, 5; ceases, 10	Missing,* 10; ceases, 16.	5	2
Methylamine	5	1	2 to 2.5	2	Slight contraction in 10.	6
Dimethylamine	5	1	Slight contraction, 4; ceases, 4.5.	2	4	1
Trimethylamine	25	2.5	Feeble, 10; ceases, 18	Missing, 15; ceases, 20	5	2
Ethylamine	5	1	Feeble, 3; ceases, 4	Missing, 2; ceases, 3	6	6
Normal propylamine.	5	1	Feeble, 4; ceases, 4.5	2 2	5	5
Isopropylamine	5	1	Feeble, 4; ceases, 5	Missing, 3; ceases, 3.5	1	1
Tetraethylammonium hydroxide.	5	No color change	Feeble, 5; ceases, 8	Missing, 2; ceases, 4	1	Missing, 2; regular, 5.
NaOH	10	No color change	Muscles contract when impulse passes. Very feeble, 10.	Occasional, 3; ceases, 4	1	1
KOH		No color change	Feeble, 8	Ceases, 6		
$Sr(OH)_2$		No color change	Feeble, 7	Ceases, 4.5		

* "Missing" indicates that sometimes the impulse can pass from A to C, sometimes not.

The actual times vary somewhat in different experiments, but the above is typical. The results of interest in the above table may be summarized as follows:

The weak alkalies (first seven) enter almost instantly, yet contractions and conduction do not cease till some time *after the color change occurs.*

The strong alkalies (last four) do not enter until *long after contraction and conduction have ceased.*

In ammonia and trimethylamine the muscles regularly lose their power of contraction before the nerves. In the remaining alkalies the nerves cease to function before the muscles as direct stimulation shows. The phenomenon is more marked with the inorganic hydroxides. Thus far these alkalies are the only substances known which affect the nerves first and the muscles afterwards.

Recovery of both contraction and conduction will take place if the tissue is removed to sea-water soon after stoppage of contraction or conduction occurs. Occasional exceptions to this rule have been recorded in which the nerve-tissue has been so injured in one spot that the impulse was unable to pass. The red color returns only in the tissue exposed to the *weak* alkalies.

Recovery from the effects of the *strong* alkalies never occurs if the tissue is allowed to remain in the solution long enough for the color change to take place, nor does the red color return on transfer to sea-water.

Ammonia and trimethylamine are less toxic than the remaining amines. The strong alkalies take an intermediate position between the two groups of weak alkalies as regards toxicity for *Cassiopea* muscle.

That the resistance to the entrance of neutral red is a property of the living cell may be shown by treating the *Cassiopea* strips with chloroform-saturated sea-water. The cells are killed and the red-stained granules are cast out in a slime from which the neutral red dye slowly diffuses. If such a strip is placed in N/250 NaOH, the slime is turned yellow in less than two minutes, *i. e.*, just as rapidly as in N/250 NH_4OH. To a certain extent the slime prevents free access of NaOH to the tissue. When stripped off with a camel's-hair brush, the underlying tissue, still pinkish in color, is rapidly turned yellow by the alkali. The slime forms most rapidly in the strong alkalies, much less rapidly in the stronger amines, and very slowly in NH_4OH and trimethylamine.

In the following experiments on tissues other than those of *Cassiopea* and on eggs, the effect of NaOH has always been compared with that of NH_4OH, for two reasons: (1) My general experience indicates that NH_4OH may be taken as a representative of the weak alkalies and NaOH as a representative of the strong. (2) Since NaOH always affects the cell before it enters, we might consider the visible alteration in function a more delicate test for the presence of the OH ion within the cell than the neutral red indicator. The control experiment with NH_4OH shows that this is not the case. The color change *always begins* and in many cases is complete before any alteration in function occurs. Even though NH_4OH enters readily it is too weak an alkali to affect the cell immediately.

PENNARIA TIARELLA.

The tentacles were stained in neutral red and the time for color change was compared with the time required for spontaneous movement to cease. Globules much like those occurring in *Cassiopea* take up the dye. They are present in all parts of the tentacles. It is probable (but can not be stated positively) that stained granules are present within muscle-fibers.

In NaOH of any concentration movement always ceases long *before* any red granules are turned yellow.

In N/1000 NH_4OH movement ceases in about 15 minutes. The red begins to change, but is not completely yellow at this concentration for a long time. In N/500 NH_4OH movement ceases before the color change is complete.

The above results were also obtained using the gill muscles and tentacles of an unidentified *Amphitrite*-like annelid from Tortugas, Florida.

SALPA DEMOCRATICA.

Red-stained granules undoubtedly occur within the muscle-fibers of the circular bands of this animal.

In NaOH of any concentration movement ceases *before* the color change.

In N/1000 NH_4OH contractions cease in about 15 minutes. The color change begins in 5 minutes, but is not complete even after an hour. In N/500 NH_4OH contractions cease before the color change is complete.

CILIA AND MODIFIED CILIA (CTENOPHORE SWIMMING-PLATE).

BERÖE OVATA.

Granules stained in neutral red occur in the swimming-plate cells, not in the plates themselves.

In NaOH the movement always ceases long *before* the color change occurs.

In N/500 NH_4OH, the plates cease beating at first, begin again in less than a minute, but have mostly stopped in 5, and all by 10 minutes. The red granules are only partially yellow even after 45 minutes, although the color change begins in the course of 3 to 4 minutes.

TOXOPNEUSTES LARVÆ.

Red granules occur in the ciliated cells, but not in the cilia themselves.

In NaOH the cilia always stop *before* the color change occurs.

In N/500 NH_4OH color change occurs in 1 to 2 minutes. Movement is stopped at first, begins again in about 4 minutes, continues slowly after half an hour, and has not entirely ceased even after an hour.

A similar result is obtained with palolo trochophores (*Eunice fucata*).

These experiments with marine cilia agree in every way with those obtained on *Paramœcium.*

The above results taken as a whole admit of only one interpretation. If NaOH prevents the normal functioning of many different types of cells without appreciably entering those cells, we must conclude that its effect is on the surface and that the alteration in function is connected with a change in the surface layer. Muscle contraction, nerve conduction, and the movement of cilia and modified cilia (swimming-plates of the ctenophores) must all be dependent, primarily, on changes in the surface layer of the muscle, nerve, or ciliated cells.

CYTOLYSIS OF MARINE EGGS.

Cytolysis of marine eggs is accompanied by (1) an increase in volume of the egg as a whole; (2) in many cases a solution of the yolk and pigment granules.

Two quite distinct views have been held as regards the cause of the swelling: (1) Swelling may be due to an increase in permeability of the egg surface-membrane such that the salts of sea-water can readily pass in; they therefore no longer balance the osmotic pressure of substances within the egg and the egg swells; (2) swelling may be due to a breakdown of protein, lipoid, or other substances and the development of a higher osmotic pressure or a swelling pressure within the egg. According to the first view, the action of a cytolytic substance must be chiefly on the surface; according to the second, action is on the cell contents. The relation of cytolysis to penetration of alkali is therefore of special interest as furnishing evidence in favor of one or the other theories of cytolysis. Details of typical experiments are given below.

TOXOPNEUSTES VARIEGATUS (MATURE EGG).

N/80 NaOH—Egg remains in solution absolutely unchanged for 6 minutes; diameter 5.5 units;[25] then swells suddenly to 7.0 units. Color change is coincident with swelling.

N/80 NH_4OH—Color change instantaneous. Egg remains in solution for 15 minutes unchanged; diameter 5.5 units. In the next 5 minutes the egg begins to appear coarsely granular and at the end of 2 more minutes has swollen to 7.5 units and is filled with a mass of globules. Swelling is relatively rapid.

EUNICE FUCATA (PALOLO WORM) MATURE EGG.

N/80 NaOH—Egg remains in solution unchanged for 8 minutes; diameter 19 units. Then swelling begins and continues slowly for 5 minutes; diameter 21.5 units. The color change occurs gradually during the swelling.

N/80 NH_4OH—Color change instantaneous. Egg remains unchanged in appearance and size and only becomes broken up into oil-like globules after an hour.

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[25] A unit is 19.2 microns.

HOLOTHURIA FLORIDANA (IMMATURE EGGS).

N/80 NaOH—Eggs remain unchanged in size and appearance for 6 minutes; diameter 14.2 units. The surface layer then becomes clear, the clear region extends inward, and in the course of 1.5 minutes more the whole egg is clear and swollen to 16 units. The color change occurs as the egg contents become clear.

In N/160 and N/320 NaOH the color change occurs some time before the egg clears and swells. Thus in N/160 NaOH the color change occurs in 18 minutes and cytolysis in 40 minutes.

N/80 NH_4OH—Color change is instantaneous. Eggs remain the same in appearance and size for an hour; diameter 14.4 units. Then swell to 18 units in the course of 2 to 5 minutes.

The immature eggs of *Fissurella* sp., *Eurythoe* sp., and an *Amphitrite*-like worm behave as do those of the palolo.

It was often noticed that if segmenting eggs were placed in NaOH different blastomeres were entered at very different times by the alkali. Thus the resistance of each individual blastomere is specific and on division the daughter cells may become perfectly distinct from each other so far as permeability relations go. Whether such differences are related to the hereditary potencies of the individual blastomeres was not determined.

It will be seen from the foregoing observations that several types of eggs may be distinguished as regards penetration of NaOH and cytolysis, viz: (1) Eggs in which cytolysis and color change are both simultaneous and rapid (*Toxopneustes* and sea-urchin eggs in general); (2) eggs in which cytolysis and color change are simultaneous but gradual (*Eunice* and other annelids); (3) eggs in which color change may occur *before* cytolysis (*Holothuria floridana*, immature eggs).

In addition to the above: (4) eggs in which the color change occurs after cytolysis (*Cumingia*, observed at Woods Hole, Massachusetts).

In *all* types ammonia and the amines enter instantly, long before cytolysis occurs.

Only one egg thus far examined (that of *Cumingia tellinoides*) belongs to type 4, and one egg only (that of *Holothuria floridana*) to type 3. In other cases penetration of NaOH and swelling are simultaneous.

Leaving aside for the moment the egg of *Holothuria*, which forms an exception to the rule that cells are visibly affected before or at the moment of entrance of NaOH, let us analyze the results obtained with eggs of types 1 and 2.

The rapid color change in ammonia shows how small the concentration of OH ions need be to affect the red-stained granules. We may assume, then, that if even a trace of NaOH entered the color change would be rapid. Since practically all marine eggs remain quite unchanged in size and in color (of the red-stained granules) in NaOH solution for some time and only after a definite interval begin to swell and to change color relatively rapidly, we may conclude that the NaOH can only force an entrance after fundamentally changing the normal nature of the cell-surface. The manner in which cytolysis is connected with such a change in the cell-surface may be conceived as follows:

The NaOH combines with substances, probably with proteins at the surface of the egg, forming a compound through which it may pass; *i. e.*, the surface is made permeable to NaOH. This compound must also allow the salts of sea-water to pass readily; the egg is likewise made permeable to the salts of sea-water.[26] Osmotic equilibrium is upset and the egg absorbs water and salts. Breaking up of the granules is due largely to the increase of water in the egg. All marine eggs swell when placed in fresh water and when the increase in volume reaches a certain value the contained granules break down; true cytolysis results. The breaking-up of the granules by distilled water is obviously not due to any chemical substance, such as alkali, entering the egg. An increase in permeability might result in swelling without the entrance of the cytolytic substance. Such is the case when *Cumingia* eggs are placed in NaOH.

However, I am inclined to think that the presence of a sufficient number of OH ions within the egg may aid in the breaking down of the granules and that this breaking down increases also the degree of swelling of the egg. Cytolysis in *Holothuria* appears to be largely of this type, since here NaOH enters before the increase in volume begins.

From this point of view both theories of cytolysis contain an element of truth. Swelling of marine eggs is due both to an increase in permeability of the surface and also to the breakdown of lipoid or protein granules within. The latter tends to increase the swelling pressure or the osmotic pressure of the egg, but is secondary to the increase in permeability of the surface.

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[26] McClendon (Am. Jour. Physiol., vol. 27, p. 240, 1910) finds the electrical conductivity in sea-urchin's eggs to be greatly increased after cytolysis, a result indicating ready passage of the salts of sea-water.

IX.

PHYSIOLOGICAL STUDIES ON CERTAIN PROTOZOAN PARASITES OF DIADEMA SETOSUM.

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PHYSIOLOGICAL STUDIES ON CERTAIN PROTOZOAN PARASITES OF DIADEMA SETOSUM.

By Merkel H. Jacobs.

INTRODUCTION.

It has been shown by the author and others that different species of protozoa have certain physiological characteristics, often almost as striking as their morphological ones, and which are probably of considerable significance in the interpretation of their habits of life and their relation to their environment. The study of such characters in the past has been greatly neglected on account of the general belief of biologists that they are exceedingly inconstant and subject to modification by external factors and therefore of little value in an understanding of the fundamental nature of the organisms possessing them. Previous attempts to detect characteristic physiological differences between different forms have for the most part been confined to organisms whose general habits of life are so dissimilar as to leave room for the objection that the differences observed may have been due merely to the effect on the animals of the different environments to which they have been accustomed and not to any innate peculiarities of the organisms themselves.

It occurred to the author as desirable to test, if possible, a series of forms which naturally live under essentially the same environmental conditions, and which may be assumed to have done so for many past generations, in order to see whether they show greater likenesses than a number of forms selected at random, or whether each has preserved its individuality in spite of the similarity of its environment. In the case of a number of parasitic protozoa living together in the same organ of the same host, we have a favorable opportunity for such a study. Even here we can not assume that each form has exactly the same environment, but, there can be no question that this environment is far more similar than that of a number of selected free-living forms. Furthermore, in order to become parasites of the same host the nature of the forms in question must have been more or less changed. In the case of intestinal parasites, for example, it would be necessary for them to acquire a resistance to the digestive juices of the host and to become accustomed to a more or less anaerobic habit of life, etc. Such changes would necessarily be in the same direction for all of them. If, therefore, a number of parasitic forms, which have had to meet and adapt themselves to the same new conditions and live under them together for thousands of generations, still show as characteristic physiological differences as free-living forms, it is good evidence of the fundamental nature of these differences. Is such the case? The present paper is a preliminary consideration of this question. The work on which it is based was performed at the Tortugas Laboratory of the Carnegie Institution of Washington during the summer of 1910.

MATERIAL.

The material used in these experiments consisted of four species of ciliate protozoa obtained from the alimentary tract of the large black sea-urchin, *Diadema setosum*, which can be procured in practically unlimited quantities from the coral reefs about the Tortugas. This form is particularly favorable as a source of material, not only on account of its abundance but on account of the high percentage of infection shown by it with respect to the protozoa in question. Out of over 100 adult Diademas examined during the progress of the work, not a single one failed to show one or more kinds of protozoa in the intestine, which often fairly swarmed with them. Doubtless the large size of the intestine, its pouch-like recesses in which stagnation of the food can occur, and the character of the food itself are factors favoring the presence of protozoan parasites or, perhaps more correctly, protozoan commensals, since none of the forms in question seem to be injurious to the host or to live at its expense, except in so far as they use up a small portion of its practically unlimited supply of food.

The forms studied were of four kinds. Since apparently none of them have been previously described or named, they are for convenience designated in the account that follows by the letters, *A*, *B*, *C*, and *D*. Of the four, *B* is the most regular in its occurrence, having been found in 100 per cent of the adult Diademas examined. It is a large, heterotrichous form allied to *Metopus*, with a hook-like anterior end and showing considerable individual variation in appearance.

The form next in abundance is *C*, which is found in about 75 per cent of the cases. It is a medium-sized, holotrichous form, elongated in shape, with the anterior end of the body transparent and longitudinally striated and the remainder filled with refractive spherules. A nucleus is visible about the middle of the body and no mouth opening can be detected.

The next form in importance is the one designated as *D*, which is found in 40 per cent of adult Diademas. It is flat and leaf-like in form, with the body slightly concave on the ventral and convex on the dorsal surface. The one margin of the body is almost straight and the other curved, giving the animal an asymmetrical shape. At the posterior end is a spur-like projection. Evidently this form is allied to the holotrichous genus *Dysteria*.

The form which occurs in the smallest number of cases (33 per cent) is *A*. When present at all, however, the number of individuals is generally very large. In size it is rather small and in shape elliptical and flattened.

Very little could be made out of its structure, owing to its small size, rapid movements, and its peculiar tendency to disintegrate with almost explosive suddenness when kept under observation on a microscopic slide. In addition to these four characteristic forms, several other ciliates were observed more rarely, and on one occasion an *amœba*. They were not found frequently enough, however, to be considered in the experimental part of the work. So far as the present observations go, the important group of flagellates appears not to be represented by parasitic forms in *Diadema*.

The parasites of *Diadema* seem to occur exclusively in the alimentary tract, none being found in the body-fluid so long as the intestine is uninjured. In the alimentary tract itself the number is not the same in the different regions, some parts being more favorable than others. The greatest numbers are found in the distended pouches of the second, or upper, coil of the intestine. In the lower coil they seem to be almost or entirely absent, even when present in great abundance elsewhere. It is interesting to note in this connection that free-living forms (*e. g.*, *Foraminifera*, etc.), which are taken in with the food, are invariably dead before the second coil is reached. The rectal portion of the intestine also shows very few protozoa, form *D* being the one most frequently encountered there, though *B* and *C* may occur rarely and in small numbers. On several occasions cysts, possibly of *D*, were found in this region. It seems quite probable that cysts thus formed may pass out and eventually infect other Diademas. It does not always happen that the different protozoa present show the same distribution, thus, *A*, for example, may be quite absent in some of the pouches and present in others, while *B*, *C*, and *D* show similar irregularities. It is usual to find several of the forms together, but there are no constant combinations; any form may occur associated with any of the others.

The above remarks concerning the abundance and distribution of the parasites apply only to adult Diademas. As might be expected from our knowledge of other animals, young individuals contain either no parasites at all or at most but a few. The youngest ones examined (0.5 inch in diameter) contained nothing. Somewhat older ones (1.5 inches in diameter) contained sometimes nothing at all, sometimes large numbers of a form not found in any of the adults examined, and in a few cases small numbers of *C* and *D*. It is rather striking that form *B*, which appears to be present in all adults, should be so constantly lacking in younger individuals. Whether this is because conditions for its existence are unsuitable or simply because no opportunity for infection has occurred could not be determined.

A search for the parasites in question was also made in sea-water and in other animals, related and unrelated to *Diadema*. The only situations in which any of them were found was in the alimentary tract of other sea-urchins, where they may occur in varying numbers. *B*, for example, was found rarely in *Toxopneustes* and *Echinometra*, and *C* on a number of occasions in *Toxopneustes*, while *D* seems to be present in every adult *Toxopneustes*, and rarely in *Echinometra*. *A* was never found outside of

Diadema. A number of specimens of *Hipponoë* were examined for protozoan parasites without results.

METHODS.

In comparing the physiological nature of the forms in question certain characters were chosen which could be expressed quantitatively and the animals examined with respect to them. Those selected all had to do with the length of time the organisms could maintain their normal activities under various conditions differing from those to which they were accustomed. The method adopted was to place the animals under the new conditions and note the time that elapsed until all visible movements ceased. In the case of ciliates this time corresponds very closely with that at which irreparable injury, resulting in death, has been done to the cell, and therefore furnishes a more or less accurate criterion of the general vitality of the organisms.

In the present experiments the number of individuals studied was always large and the range of variation within the species small, and therefore it was found possible to choose, as the point for comparison, the time at which all the individuals of the given species had been rendered motionless. This point was easy to determine and the general agreement in the results obtained on successive days by the use of the above method showed it to be sufficiently accurate in a case, such as the present one, where it was desired to express the relative and not the absolute resistance of the four forms in question. Most of the experiments were repeated a number of times with material obtained from different sources, so as to eliminate accidental errors. The characters, in respect to which the different forms were compared, were as follows: (1) Ability to live outside the body of the host; (2) ability to live in the body-fluid of other related animals; (3) length of life of the parasites after the death of the host; (4) resistance to CO_2; (5) resistance to H_2S; (6) resistance to the decomposing proteid substances; (7) resistance to H_2SO_4; (8) resistance to KOH.

EXPERIMENTS.

ABILITY OF THE PARASITES TO LIVE OUTSIDE THE BODY OF THEIR HOST.

It is well known that protozoan parasites show a varying ability to live apart from their host. In general, the less specialized the parasite and the looser the relation between it and its host, the longer it may be expected to live under conditions other than the natural ones. The forms in question are not highly specialized and, as might be supposed, are for the most part able to live for a considerable time outside the intestine of *Diadema*, though so far as these experiments go, a continued existence of this sort is in no case possible. The point of particular importance, however, from the standpoint of the present investigation, is that the four forms studied show marked differences in behavior when removed from the body of their host. If a number of food pellets from the intestine of a *Diadema* be shaken up with a little fresh sea-water, none of the parasites obtained in this way is at first visibly injured. If, however, a few cubic centimeters of the mixture be placed in a covered glass dish which prevents evaporation but which, being only partially filled, does not prevent contact of the liquid with the air, it will be seen that all of the animals present will die after a length of time which is fairly constant for each species. The first one to disappear apparently always is *A*, which is no longer present after 2 to 3 hours.

It may be mentioned that no way of keeping *A* longer than this in artificial cultures was found except to place a relatively large quantity of the intestinal contents in a test-tube with the addition of only sufficient water to keep it moist, in which case the animals retained their activity somewhat longer. *D* is next to *A* in respect to its behavior in sea-water culture, and usually is dead at the end of 24 hours, though a few individuals may survive several hours longer. *C* does not differ very markedly from *D*, but is somewhat more resistant, living on an average 30 hours or a little over. *B* is by far the most resistant form, often surviving and continuing active for 2 or 3 days. The relative ability of the four forms to live outside the body of their host may therefore roughly be expressed by the ratio 1 : 24 : 12 : 10.

To determine to what extent the injurious effects of removing the parasite from their host were due to the action on them of the oxygen of the air, a parallel series of experiments was tried in which oxygen, so far as possible, was excluded. This was done by shaking up the food pellets from the intestine with sea-water from which the air had been removed by boiling (the concentration of salts being kept constant by the addition of distilled water) and immediately placing the liquid in dishes which were completely filled, covered, and sealed with vaseline to exclude the air. Such treatment was found not materially to alter the length of life of any of the forms except *B*, which in one such experiment lived for 7 days, the longest time for which it was found possible to keep any of the forms alive by any of the methods employed. *A*, *C*, and *D* died in about the usual times, the life of *A*, if anything, being slightly shortened and that of *C* slightly lengthened. In no case except that of *C*, however, were the differences significant.

ABILITY OF THE PARASITES TO LIVE IN THE BODY-FLUID OF TOXOPNEUSTES.

Early in the course of these experiments it was observed that the forms in question live in cultures made with the body-fluid of *Diadema* about the same length of time that they do in sea-water. This is not surprising, since the osmotic properties of the body-fluids of sea-urchins and sea-water are about the same. A point of some interest seemed to be whether the body-fluid of a nearly related genus of sea-urchins would be as favorable as that of the host or as sea-water, or whether there might be present in it in addition to the salts of sea-water certain other substances of more specific nature which would have a toxic effect. To determine this point, therefore, experiments were made, but unfortunately they were not very extensive. One such experiment may be mentioned. *A*, *B*, and *C* obtained from *Diadema* were placed in a small quantity of the body-fluid of *Toxopneustes*.

A was found to be dead at the end of 1.5 hours, *B* in 3 hours, and *C* was living after 15 hours, but dead in 24. In a control culture in which the body-fluid of the original host was used in place of that of *Toxopneustes*, *A* lived about 2 hours, *B* was still active at the end of 24, and *C* died somewhere between 15 and 24. It will be seen, therefore, that *A* and *C* are less affected by the change than is *B*, which normally lives 10 to 20 times as long in the body-fluid of its own host as in that of *Toxopneustes*. Unfortunately *D* was not available at the time when this experiment was performed. The converse of the above experiment, namely, the subjecting of parasites obtained from *Toxopneustes* to the body-fluid of *Diadema*, was also tried with similar results. In this case only *D* was available, it being the only one of the four forms found in quantity in *Toxopneustes*. In the control culture in which the body-fluid of their own host was used the animals were normal after 15 hours; in the body-fluid of *Diadema* they were dead in 2 hours.

The injurious effects of transferring the parasites to the body-fluid of an animal other than their host are almost certainly not due to physical (*e. g.*, osmotic) differences between the body-fluids of the two animals, since they may be transferred with impunity from either host to sea-water, the body-fluid of sea-urchins having approximately the same osmotic properties as the latter. Furthermore, in a number of experiments in which the concentration of the liquid surrounding the parasites was purposely suddenly altered, the forms in question were shown to be quite resistant to changes of this sort. It may be assumed, therefore, that chemical rather than physical differences in the two body-fluids are responsible for the death of the animals. It is rather interesting that different races of the same species are found adapted to different hosts and apparently can not be suddenly transferred from one to the other. Where adaptive characters such as these are concerned, we can look for a considerable amount of variation within the species. But in the case of non-adaptive characters, such as some of those mentioned in the succeeding experiments (resistance to H_2S, H_2SO_4, etc.), it was found that the properties of form *D*, for example, were practically the same whether it was obtained from *Diadema* or *Toxopneustes*, and consequently that such characters are fairly constant for the species.

LENGTH OF LIFE OF THE PARASITES AFTER THE DEATH OF THE HOST.

If a dead *Diadema*, either opened or unopened, be covered with sea-water and allowed to stand in a warm room, the number of parasites present soon begins to diminish and after a variable time all disappear. Sometimes in as short a time as 12 hours none are to be found, though in other cases they may be active for 24 hours or more. In no instance, however, do they seem to persist more than 30 hours at the room temperature of the Tortugas laboratory (85° to 95° F.). They usually disappear in the order, *D*, *A*, *C*, *B*. In one series of observations *D* was dead in 12 to 15 hours, *A* in a little less than 20, while *C* and *B* survived somewhat over 24. While the absolute time of disappearance differs in different experiments the relative order for the four forms seems to be quite constant.

It will be seen, therefore, that in respect to their ability to live in the body of the host after the latter's death, just as in many other respects, they show certain characteristic physiological differences. The relative length of life of the four forms is roughly 1.5 : 2 : 2 : 1. Just what factors cause the death of the parasites under these conditions is not certain, but probably the products of decomposition present play an important part. As will be shown later, some of these substances are markedly toxic, though each of the four forms is affected differently by them and the immediate cause of death may perhaps be different in the different cases.

EFFECTS OF CO_2 ON THE PARASITES.

Carbon dioxide certainly occurs normally in the intestine of *Diadema* and therefore small quantities of it can not be markedly injurious to any of the four forms in question. With large quantities the case is different, all being killed in a fairly short time, though the four forms are by no means alike in their resistance. If a drop of fluid containing them be exposed to a continuous stream of the gas in a gas-chamber and kept under constant observation, it will be seen that *D*, the least resistant form, is rendered motionless in 3 or 4 minutes; *A* comes next, the time required for it being 5 to 6 minutes; *C* remains active for about 15 minutes, and *B* for from 45 to 60 minutes. The ratio of resistance is therefore roughly 1.5 : 15 : 4 : 1; *B* being the most resistant form and *D* the least. The same order of resistance was found to hold if instead of subjecting the animals to a stream of gas they were placed in a relatively large quantity of sea-water charged with CO_2 by means of a "sparklet" bottle.

EFFECT OF H_2S ON THE PARASITES.

Hydrogen sulphide begins to appear in the intestine of *Diadema* in considerable quantities soon after death. The vapors given off on gently warming a little of the intestinal contents taken 12 hours after death darken lead acetate paper very perceptibly, and after 24 hours the reaction may often be obtained without the application of heat at all. As this substance might be suspected of playing a part in causing the disappearance of the parasites from the intestine of the host after death, it was of interest to examine its effects on the four forms in question. As might be expected, it proved to be markedly toxic to all, but the different forms resisted it in pronouncedly different manner, and, furthermore, the order of resistance is different from that shown to CO_2. *C*, for example, which is next to the most resistant form to CO_2, is killed first when a current of the gas is passed through the gas-chamber containing the animals, the time required being only 1 to 2 minutes; *D* comes next, with a period of about 3 minutes; *A* lives 3 to 4 minutes, and *B* 10 to 15. The ratio of the resistance of the four forms is therefore approximately 2.5 :.8 : 1 : 2.

EFFECT OF DECOMPOSITION PRODUCTS ON THE PARASITES.

In an attempt to throw light on the cause of the disappearance of the parasites after the death of the host, the effect on them of the direct products of the decomposition of the tissues of *Diadema* was tried. The method used was to allow fragments of *Diadema* tissues to decay in the water for 3 days at room temperature; 5 drops of the resulting infusion were then added to 10 drops of the fluid containing the animals. The results were most surprising. *B*, which had hitherto proved the most resistant form in most of the experiments, was killed almost instantly—certainly in less than 5 seconds; *A* lived from 10 to 60 seconds; *D* 20 to 25 minutes; and *C* as long as 2 hours. *C*, therefore, which is less resistant than *B* to most conditions, is in this case many more times resistant. The ratio for the four forms is roughly 7 : 1 : 1,500 : 250. These figures seemed so remarkable that the experiment was repeated several times, but always with essentially the same results.

THE EFFECT OF H_2SO_4 ON THE PARASITES.

A limited number of experiments were tried with this substance. It was necessary first to determine the proper concentration, since if the solution is too strong death occurs so quickly that its time can not be accurately observed, and if too weak it occurs so late that other factors have time to complicate the results. A series of preliminary experiments showed that a favorable proportion is 1 drop of N/10 H_2SO_4 to 5 drops of the fluid containing the organisms. Of course, care had to be taken that the acid was immediately mixed thoroughly with the culture medium, otherwise the organisms were not all subjected to exactly the same conditions. Treated in this way, the four forms showed striking differences: *A* had a very low resistance, being killed in about 5 seconds; *D* was killed in less than 30 seconds, but the exact time (somewhere between this and 5 seconds) was not noted; *C* died in about 30 seconds; while *B* survived 1.25 hours or 900 times as long as *A*. This is seen to be a surprisingly high resistance to acid when it is remembered that *Paramecium* and many other fresh-water forms are killed almost instantly by exposure to N/500 H_2SO_4. Summarizing the results of the observations on the resistance of the four forms to H_2SO_4 we have the ratio, 1 : 900 : 6 : $<$ 6.

EFFECT OF KOH ON THE PARASITES.

This substance used in the same concentration as H_2SO_4, as might be expected from results obtained with other animals, is on the whole much less injurious. *A* (the least resistant form) was found to be killed in about 2 minutes, *D* in somewhat less than 45 minutes, *B* in about 1 hour, and *C* in 3 to 4 hours. It will be noted that while *A*, *C*, and *D* are much more resistant to KOH than to H_2SO_4, *B* is actually less resistant. The ratio for the four forms is roughly 1 : 30 : 100 : 20.

CONCLUSIONS.

The general results of the experiments performed is to show surprising differences in the resistance of the parasites of *Diadema* to various unfavorable conditions. In some cases the most resistant form may live several hundred times as long as the least resistant one. *B* is 900 times as resistant to H_2SO_4 as *A*, and the difference between *B* and *C* with respect to products of decomposition is still more striking. Furthermore, a form which is strongly resistant to one condition may be only feebly so to another, and vice versa. For example, *B* is 8 to 10 times as resistant to H_2S as *C*, but 1,500 times less resistant to certain of the products of decomposition of *Diadema* tissues. *C* is more resistant to CO_2 than *A* or *D*, but less resistant to H_2S. *A*, *C*, and *D* are 24 to 450 times more resistant to KOH than to H_2SO_4, while *B* is somewhat *less* resistant. Other similar facts could be mentioned.

Comparing all of the results obtained, it is therefore seen that the similar habit of life of the four forms in question has not brought about physiological similarity except in certain adaptive characters which are a *sine qua non* for continued existence in the same host (*e. g.*, ability to resist the digestive juices of the latter, etc.). In other respects they are just as different as almost any four free-living forms that might be selected and so far as the evidence of these experiments goes it seems to show that the physiological characters of an organism are not merely the result of its environment, but may be as fundamental and characteristic as its morphological ones.

X.

ORIGIN OF THE ELECTRIC TISSUES OF GYMNARCHUS NILOTICUS.

BY ULRIC DAHLGREN
Professor of Biology, Princeton University.

Nine plates and nine text-figures.

ORIGIN OF THE ELECTRIC TISSUES OF GYMNARCHUS NILOTICUS.

BY ULRIC DAHLGREN.

In the seven types of electricity-producing fishes the exact development by which the electric organs and tissues are produced during the creature's life is known in only two, leaving the other five unknown. It also happens that the two forms which have been studied as to the histogenesis of their electric organs are the only two elasmobranch fishes among the seven, so that we have not as yet seen how the remarkable electric tissues in *Malopterurus*, *Gymnotus*, *Astroscopus*, the mormyrids, and *Gymnarchus* are developed. Also, of the five teleost types we know the structure of the full-grown electric organs in all of them pretty well, except in *Gymnarchus*. This fish is found in Africa and has been rather rare, so that but two workers have published observations on it, both of them a long time ago and from poorly preserved material.

It was, therefore, with great pleasure that the writer came into possession of some embryos of this rare form through the kindness of Dr. J. Graham Kerr, Dr. Arthur Shipley, and Dr. Richard Assheton, to whom he wishes to express his most sincere thanks. This material was collected in Africa by Dr. Samuel Budgett some years ago and was in most excellent condition, owing to the great care and skill with which Dr. Budgett put it up and cared for it. The collecting was done unflinchingly and faithfully, under conditions of hardship and sickness that few white men could stand, and Dr. Budgett lost his life from exposure and illness incurred in part by this work. A full account of his trip and of the scientific results should be read in the Budgett memorial volume issued by Dr. Shipley, Dr. Kerr, Dr. Assheton, and others in 1907, through the Cambridge University Press (24).[1]

It is somewhat unfortunate that the structure of the electric organs in the adult fish could not be worked up at the same time that this paper was written, but the writer has material on the way from Khartoum and hopes to publish a second paper shortly.

But three papers have been published on the electric organ of this interesting fish, one by Erdl (15) in 1847 and another by G. Fritsch (19) in 1885. Rüppel's publication on the subject could not be found, but Fritsch states that Rüppel mentioned the peculiar structures which we are considering, and so he stands at present in the writer's knowledge as the first one to have seen and reported to science the electric organs of this fish, although he was in doubt as to their significance. Erdl used a specimen which was so poorly preserved and so soft that when he cut the animal across its body, the electroplaxes and connective tissue ran out of the muscle in which they are embedded. Nevertheless Erdl stated that it was probably an electric organ, coming to this conclusion by a comparison, of such features as he could make out, with the structures found in the tails of the other electric fishes, as *Raja*, *Mormyrus*, and *Gymnotus*.

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[1] The figures in parenthesis refer to the literature cited, p. 193.

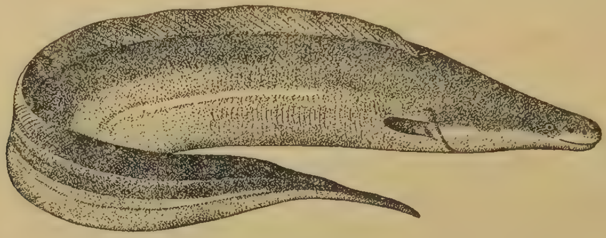

FIG. 1.—General view of *Gymnarchus niloticus*. (Drawn from a lantern slide made from a figure in Jordan's "Guide to the Study of Fishes," New York, 1905.)

Fritsch (19) in 1885 worked on better material and gave a more complete account of the anatomy of the organ, especially of the histology of the electroplax; but he came to the rather strange conclusion that it was not an electric organ at all, assuming the erroneous position that, on account of the large blood-supply, the organ acted in some way as a storage for oxygen during the period of hibernation made necessary by the drying of waters at certain seasons. Fritsch was also mistaken in calling the fibrous contents of the electroplaxes "connective tissue." He went a great deal farther than Erdl, however, in describing the gross anatomy of the electric organs and surrounding tissues.

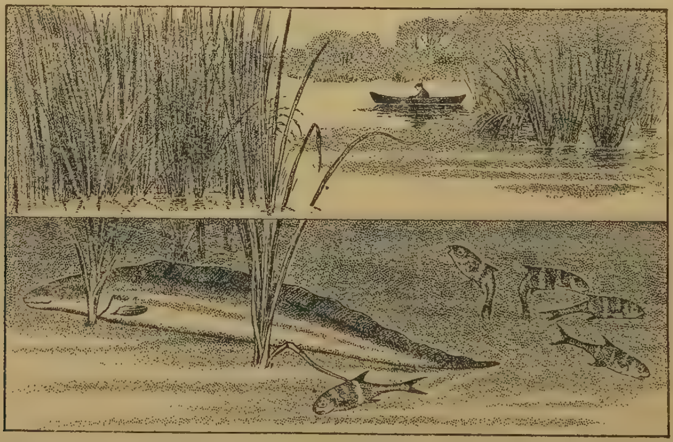

FIG. 2.—Scene drawn from descriptions to illustrate the habitat of *Gymnarchus* and its manner of swimming. (Drawn from lantern slide made from a drawing, by Bruce Horsfall.)

Not having full-grown material, the writer must rely on Fritsch's figures and descriptions for the adult gross anatomy, although the oldest embryos (about 42 days) used for this paper represent practically adult material so far as the histology of the electroplaxes is concerned. A short résumé of the adult anatomy will make a good basis for the embryological descriptions to follow.

The fish (see text-fig. 1) is a mormyrid of elongate form, so much so as to make it almost eel-shaped, although not quite so much so as the *Gymnotus* of South America. It possesses an extensive development of the dorsal fin, which extends from forward on the neck to within a short distance of the tip of the tail, where it suddenly stops, leaving the tip of the tail naked of fin; whence the name of the fish, *Gymnarchus niloticus.* This heavy fin, well provided with a series of lateral ray-muscles, is used extensively by the fish as a means of propulsion, by holding the body straight and stiff and causing a series of lateral undulations to pass from behind forward, thus driving the body backward (see text-fig. 2) or, from front to rear, which causes the fish to move with its head forward. I have not heard, but I presume that in moments of unusual effort the animal can swim by means of the common sinuous body-movements used by other elongate fishes, as is well illustrated in the eel.

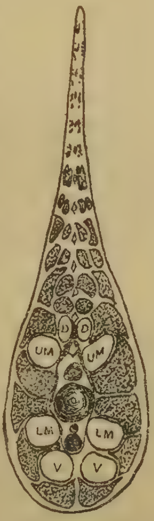

Fig. 3.—Section through mid-tail region of body of an adult *Gymnarchus*, showing position of the eight electric spindles and their relations to surrounding muscular and bony structures. *D*, dorsal spindles; *U.M.*, upper median spindles; *L.M.*, lower median spindles; *V*, ventral spindles. (After Fritsch.) × unknown.

The posterior tip of the creature's body is interesting. As has been mentioned, this end is free from the fin for some distance (see text-fig. 2). Also it is round in section and ends bluntly. When swimming backwards the animal uses it like a finger to feel its way. The peculiar round and blunt end may be explained by the fact that this tip contains the largest and best-developed portion of the electric organ, which fills the lateral parts of the body at this point almost to the exclusion of the ordinary muscle.

As Erdl and Fritsch have described, the electric organ consists of eight long "tube-like" or cylindrical structures, four on each side, embedded in the muscle tissue as close to the median bony parts as a little connective tissue in between will permit. Four of these are present on each side (see text-figs. 3 and 4), and they may be called in order from above downward, the *dorsal*, the *upper middle*, the *lower middle*, and the *ventral* cylinders, or spindles, of the electric organ. In a section cut through the body at a point midway between tail-point and anus (see text-fig. 3) the dorsal spindles are to be found, just above the union of the neural spines of the vertebræ, and set closely together with only the dorsal spine and some connective tissue between them. The upper median spindles are more widely separated by the neural canal and its contained spinal cord. This second pair of spindles are at the level of the upper part of the spinal cord. The lower median spindles are found much below, on each side of the ventral processes of the vertebra. They lie at about the level of the caudal artery and almost as widely separated as the upper median pair. Lastly, the ventral pair of spindles are found just below the latter pair and slightly below the level of the caudal vein, which lies between them. They are separated a little less than the lower median pair by the narrower bony structures at this point.

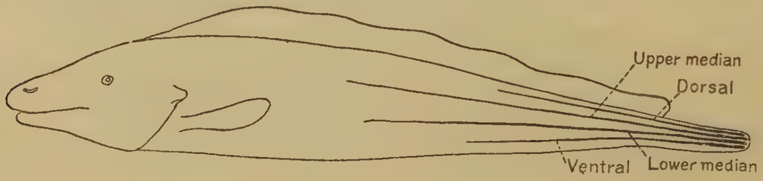

FIG. 4.—Diagram showing longitudinal position and extent of electric spindles in *Gymnarchus*. Those of one side only can be shown in figure.

The four spindles are largest in diameter in the tail, especially out in the thick, finger-like, naked extremity. From this part they taper to a smaller size as they go forward in the body and they finally become thin and end at points in the neighborhood of the anal opening (see text-fig. 4). All are not of equal length. As Fritsch has shown, the dorsal organ is the shortest and extends for about 20 cm. in a fish of 89 cm. length. The ventral pair reach for about 5 cm. further, or 25 cm. in length in a fish of the same size. The two median spindles reach for about 40 cm. from the tip of the tail.

Each spindle is marked off clearly from the neighboring muscle, and other tissues which are found next to it, by a distinct connective-tissue covering. In my largest specimen, which is a young fish of 40 days, this is well shown and is exactly like other dividing connective-tissue sheaths that surround the various muscle divisions. Like them, it often contains pigment cells which show golden-brown pigment granules.

The important contents of these spindles are alternate, cylindrical segments of a denser, deeper-staining, muscle-like substance, the electroplaxes; and a connective tissue of jelly-like, grayish transparence which in all ways appears to be similar to the "electric connective-tissue" found between the electroplaxes in the other electric fishes. In this tissue are found the blood-supply, which is largely in contact with the ends of the electroplaxes, principally the anterior end; and the nerve-supply of thick, medullated fibers which run towards, and are attached to, the posterior ends of these organs. In this case, according to both Pacini's law and the fish's relationship to *Mormyrus*, the direction of the current at time of discharge should be from tail towards the head. I have been unable to learn, from the literature of travelers and scientific collectors and observers, if the electric discharge is strong enough to be felt by the hand. Budgett does not mention it, and no other does, so I conclude that it is not a strong shock and that the organ must be classed with the weak electric organs, as is the case with its relatives, the various mormyrids. The natives of Africa are much afraid of the creature, especially at nesting time, and one of its Arabian names, "Abu rhad" meaning "father of thunders," might seem to indicate perceptible electric powers.

The embryos and young fishes put at my disposal by Dr. Kerr and Dr. Assheton were five in number, and of these three were the suitable stages from which this paper was worked out. The significant development of the electric tissues in *Gymnarchus* takes place between the ninth day of embryonic life, at which time the embryo possesses a fully formed and complete musculature in the tail with no sign of an electric organ, and the fortieth day of development, at which time it can be seen that the embryo has developed its electric organs, out of a certain part of the previous musculature in the tail, to a degree that shows the farthest advanced electroplaxes in a practically adult condition.

The most interesting and critical stages in this metamorphosis of muscle into electroplax appear to take place within much closer limits, and stages from the eleventh to the fifteenth day would include them. These significant changes have been studied and drawn principally from an embryo 12 days of age, fixed in sublimate-acetic, and showing the changes very much to my satisfaction. A point of interest and importance in this study is that, in earlier embryos, the myotomes and electric spindles are youngest, least developed, and growing fastest in the posterior part of the body or nearest the tip of the tail; while in older embryos and in the adult the greatest, most complete, and most characteristic development of the electroplaxes is to be found in the end of the tail or at the posterior end of the spindle. Thus the adult structures in the anterior part of the spindles represent a somewhat inferior and less complete change of the muscle tissue into electric tissue than the posterior parts of the same organs do.

The same importance attaches to the fact that the rates of development of the several spindles seem to vary. The lower median spindle starts first to differentiate, extends farthest forward in the body, is larger than the others when developed, and during early development is always in advance of the corresponding parts of the other spindles. The upper median spindle closely follows the lower. The ventral spindle is much behind the two median ones, while the dorsal spindle represents the latest and weakest development and is shorter than any of the others. These facts have made it possible in the present study to get many stages of development from very few embryos.

STUDIES OF AN EMBRYO NINE DAYS OF AGE.

This little fish was 26 mm. in length and, while the egg-membrane had been ruptured and cast away, the animal was still forced to remain in its nest because of its huge, elongate yolk-sac, still unabsorbed, and because of its otherwise undeveloped organs of alimentation, locomotion, etc. The posterior part of the body was carefully cut into four portions (see text-fig. 5) to be sectioned as follows: First, from in front of the anus to a point about 26 vertebral segments posteriorly. The last three segments of this portion were sectioned transversely and serially (region A_1) while the remaining anterior part was sectioned vertically and longitudinally (region *A*). Another portion of 19 vertebral segments further caudad was removed and its posterior two segments sectioned transversely (region B_1), while the anterior part was cut as before in vertical, longitudinal sections (region *B*). A third part, of 18 more vertebral segments, was treated in the same way, except that no transverse sections were taken and the entire piece was cut vertically and longitudinally (region *C*), while the remaining portion or tail-tip was cut serially in transverse sections and forms a series (region *D*).

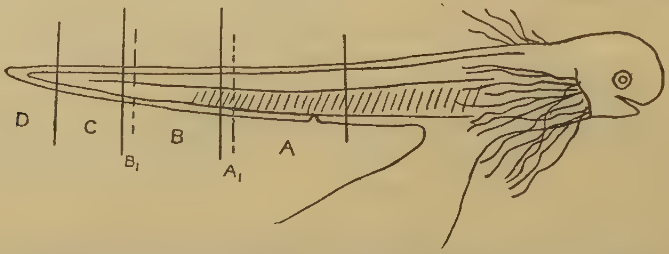

FIG. 5.—Outline of body of an embryo of *Gymnarchus* nine days old. Transverse lines and letters indicate parts sectioned for study. For explanations see text. (Copied from Assheton in "The Work of John Samuel Budgett.") × 5.

Figure 1, plate 1, shows a transverse section through the body a rather short distance from the extremity of the tail or at a point where we are sure that the electric organ will be well developed a little later in life. It may be thought that such a section could be taken for study to better advantage in a more anterior position on account of the earlier anterior development just discussed, but it must be taken into account that the tail segments are being added and are still growing rapidly at this age, that they are very short and very crowded, and therefore the location of this section is, in reality, fairly well forward in the future electric spindles. Conditions were much the same in region *C*.

This section shows a good development of muscle fibers as indicated by the shaded area. As is usual in vertebrates, the most advanced stages of muscle-cell development are to be seen at the lateral periphery of the myotome. Here, at the point indicated in figure 1, plate 1, by the dotted line, a layer of the outer muscle cells, two or three deep, has acquired an average of about 26 myofibrils (an average of 14 counts). These are grouped in one (fig. 2, plate 1, *B* and *C*) or sometimes two bundles in the cell (fig. 2, plate 1, *A*) and their correct spacing and their thickness and staining power indicate muscle cells of normal development and good functional activity. The remaining and inner muscle cells of the myotome, forming its larger bulk, show, as one examines them successively farther inward (toward the median line), a series of earlier stages, until at many points on the inner edge of the myotome the smallest cells are seen with large nuclei and no myofibrils at all (fig. 2, plate 1, *C*). These youngest cells are particularly abundant at the dorsal and ventral edges of the myotome.

From what we know of the position of the future electroplaxes and their relation to the muscle-masses, we can be sure that it is from the inner edge of the myotome that the electric tissue is to come, and a close scrutiny of the cells which form this edge shows that at two points only is there any indication of such a development.

One of these points, marked with a circle (⊕) in figure 1, plate 1, is where the two myotome segments (dorsal and ventral) meet and close in against the notochord. Here we see, in some of the sections, several especially large and strongly developed fibers, somewhat detached from the rest of the myotome and resting against the vertebral disk. Several reasons exist, however, why these fibers do not represent the future electric organ. First, they are in the exact median position which remains constant during growth and in which no electric tissue is to appear. Second, they are very short and are not attached to each other longitudinally, as other fibers are, by means of connective tissue, but are attached to the bodies of the future vertebra. They may be called the vertebral fibers.

A second point can be seen where some muscle-tissue shows unusual development. At this point (marked with a circle (○) in fig. 1, plate 1) are several muscle cells which show from 10 to 18 myofibrils each, and a degree of development almost equal to the cells in the outer layer. These cells are represented as seen under high magnification in figure 3, plate 1. They are, I believe, destined to be the future electric cells, several of which will unite and form one of the electroplaxes of the lower median spindle of the electric organ. I base this assertion only on their position and their somewhat advanced development as muscle fibers, for they show at this time no indication, other than their size, of developing into electric tissue.

Their position is slightly too far dorsal for the lower spindle, in an adult *Gymnarchus*, but when we examine the 12-day-old and 42-day-old stages, we find that the normal growth of the muscle-mass will carry this point to exactly the proper position for that spindle to lie at, in the grown fish.

An important point in studying these cells in figure 3, plate 1, is to note that they are separated from the rest of the myotome and from one another by other muscle cells of weak or of earlier development and containing only a few myofibrils. These weaker cells, and even some of the more advanced ones, are destined to degenerate during the development and growth of the electroplax. In figure 3, plate 1, 7 cells are present that will probably take part in the formation of the electric spindle at this point. Some of them are contiguous and others are widely separated.

In figure 4, plate 1, we have a fortunate longitudinal section from the adjacent region of this same embryo. The section shows in longitudinal view, and under low magnification, the same group of muscle cells marked with the circle (○) at both ends. Also, it shows a portion of the ventral part of the myotome with its strongly developed outer layer (*o.f.*). The vertebral fibers mentioned above are not visible, but their position in a few following sections is indicated by a line one can imagine to be drawn between the points marked with the figure ⊕. It has been considered unnecessary to figure the myofibrils longitudinally under large magnification. The cross-striation is very plainly visible and is the same in all parts. Each of several regions of this embryo was examined and in all its parts were the same conditions found. We may sum up by stating that the embryo of 9 days age shows no electric tissue and but a very weak indication of the development of such a tissue, all of the myotome cells being decidedly of the muscular type. Our only evidence is drawn from future stages, as to the position of such electric tissues and from the otherwise unexplained precocity of the fibers in our 9-day embryo, in that same position.

STUDIES OF AN EMBRYO TWELVE DAYS OF AGE.

This animal (text-fig. 6) showed a considerable increase in size, not so much in length as in thickness. The posterior part of the body of this specimen was divided into the following pieces: First, from a short distance behind the anus to 14 vertebral segments further caudad. Two-and-one-half segments were cut off of this by serial, transverse sections from the posterior end (region $A-1$) and the remainder was cut into vertical, longitudinal sections from right to left (region *A*). Second, 15 segments more were removed and 3 segments of the posterior end were again cut as serial, transverse sections (region B_1) and the remaining 12 segments were cut serially in horizontal, longitudinal sections (region *B*). Third, 14 segments were again cut off and the posterior 3 segments were cut transversely (region C_1), while the remainder was cut in vertical, longitudinal sections (region *C*). The next 18 vertebral segments formed a piece that was cut in vertical, longitudinal sections (region *D*), without any transverse sections being made, and this left a small bit of somewhat curled tail-tip, which was so young in development that its segments could not be easily counted. This is cut transversely in series (region *E*).

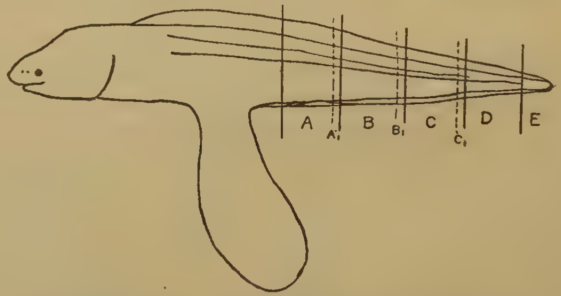

FIG. 6.—Diagram of body of an embryo of *Gymnarchus* 12 days old. Lines and letters indicate the regions studied. For explanations see text. (Copied from the same source as fig. 5.) × about 3.25.

Study of this embryo may best begin by examining a transverse section through the posterior part of the body (fig. 5, plate 2) to see what has become of the inner parts of the myotomes. Looking first for the point *L.M.* as seen before in figure 1, plate 1 (marked with ○), we can see at once that it is present as a compact mass of muscle-like tissue now clearly separated from the rest of the myotome. Further, there are to be seen on each side three other similar sections of the same muscle-like tissue, all more or less also separated from the main mass of the myotome. The most important part of our study now consists on the one hand in proving that this mass *L.M.*, in figure 5, plate 2, is derived from the undoubted muscle-fibers (marked with ○), as seen in figure 1, plate 1, and on the other hand in showing that this same structure is to become the finished electric tissue as seen in such advanced development as in figure 21, plate 8, for instance.

In tracing it back to the muscle, we are much assisted by the fact that the dorsal and ventral spindles are always in an earlier or in a less complete stage of development than the upper middle spindle, or, particularly, the lower middle spindle under consideration. And, since in this embryo of 12 days the development has gone to considerable length, and since a slightly younger stage, say a 10- or 11-day embryo, was not included among the embryos at my disposal, this fact is of much importance, because it will be fair to take the left ventral spindle as an intermediate step in the comparison. Figure 9, plate 3, is a highly magnified section of the locality of the left ventral spindle from region *C* in the embryo of 12 days, and in it we can see a mass of muscle-like cells, closely associated and lying at the inner edge of the myotome. Certain changes clearly differentiate them from the rest of the muscle, however. One change is the fact that the myofibrils have shown a large diminution in size or thickness and have also suffered in power to take the stain; particularly on the periphery of some of the muscle-columns they almost refuse to take it. Their spacing is also irregular and they show a distinct tendency to clumping together and, in some cells, to get close to the nucleus or even to surround it. They can be readily compared in figure 9, plate 3, with the well-developed young muscle cells just outside and to the left of them. The dotted line marked *XX* indicates a separation of the two. All of those to the right of this line show the condition of the myofibrils mentioned above; those to the left show the usual condition of muscle cells of this age in fishes.

A second characteristic of the changing muscle cells under discussion is in their cytoplasm. It appears more abundant, although this may be due to the smaller fibril bundles. But it also stains more heavily with such stains as eosin, erythrosin, and orange G. With the eosin, for example, it also shows a more yellowish tinge than the cytoplasm of the usual muscle cells in the same sections. And lastly, some of the muscle cells seem to have entirely disappeared or to have greatly shrunken. This latter fact causes a loose and separated condition to obtain among the metamorphosing cells which shows in sharp contrast (fig. 9, plate 3) to the compact condition seen in the typical muscle cells to the left of or outside of the line *XX*.

Another important fact can be seen among the changing cells in figure 9. Those near the center of the group show a tendency to touch or coälesce with each other. Already at this early stage this marks a difference in the group. Those cells within the dotted circle are destined to unite with one another in a compact bundle to form the future electroplax; while those of the group which lie outside of the dotted ring are to degenerate and atrophy altogether, in order to make room for the growing electroplax. Just mediad of, or to the right of, the dotted ring is seen a cell that has entirely lost its myofibrils and whose cytoplasm is vacuolated and about to be absorbed. At other points, to the left, still other cells can be seen that have dwindled to a smaller size than any of the normal muscle cells. The cells within the ring are judged by the writer to be those which will form the electroplax at this point, because they are starting to unite and, also, because they occupy the position at which the electroplax will lie. Another reason is that their myofibrils are weakening and clumping. Some of those cells lying inside of the ring may also atrophy. This can not be infallibly judged; but certainly all of those outside of it and to the right of the line *XX* are about to degenerate and are not found in later stages.

The connective tissue creeps into the neighborhood of and among these metamorphosing muscle cells at this time, and good mitotic figures can be seen, showing that it is increasing the number of its cells. It does not, however, penetrate the groups of future electric cells inside the dotted line. Also blood, pigment, etc., are to be seen in characteristic positions.

A transverse section of the body at region B_1 need not be illustrated at this point by a low magnification figure, because it is so like figure 5, plate 2, in general appearance. But it happens that in such a section several very interesting stages, forming a sequence of which figure 9, plate 3, can be taken as the first member, were noticed, and figure 10, plate 3, is the second in this series. This drawing represents the right ventral spindle in region B_1, and a number, five to be exact, of the transforming cells can be seen here in closer union than the corresponding cells were in figure 9, plate 3. One or possibly two of these may disintegrate a little later. A dotted ring is not necessary, because the connective-tissue cells have partly marked off the electric cells, and outside of this incomplete ring and above it can be seen two muscle cells which are atrophying. A third cell is shown in a final stage of disintegration. Its cytoplasm is almost clear, or all gone, and its myofibrils have united in a single lump, which will soon become a round droplet or cell-inclusion that will afterwards disappear.

Moving to the right upper middle spindle (fig. 11, plate 3), we need very little explanation to see how the four or more muscle cells that first composed this structure have come into a still closer union. Below and to the right (outer) are still seen some of the degenerating muscle cells—five in this figure.

Several important points must be discussed in connection with this figure; the component cells, as seen here, are not simple, single muscle fibers. The lower one can easily be seen to have two myofibril bundles as well as two nuclei. The presence of two widely separated fibril bundles, as well as the large size of the cell, makes it nearly certain that the structure was formed by the coalescence of two muscle fibers. In the upper region of the future electroplax, however, a large cell is seen which has only one fibril bundle and four nuclei. It is possible here that this was one cell and that it is growing in size and multiplying its nuclei by amitotic division. As this is the same process which goes on in ordinary muscle cells of this age, it is not surprising to find it going on here, and in older specimens we shall find it the rule. The cytoplasm of all these electric cells is abundant at this stage and is dense staining with the acid dyes.

As compared with figure 11, plate 3, the next illustration, figure 7, plate 2, is most interesting and is a step of some magnitude in the development of the electroplax. The cytoplasm of such cells as compose this young electroplax is all united into a single mass and the relation of nuclei to fibril bundles is completed. The nuclei are always peripheral and the fibril bundles appear to form a single central mass. This is the permanent condition which will obtain throughout the life of the organ, and is also the condition common to some other electroplaxes, as, for instance, *Raja* and *Mormyrus*.

Just how the several fibril bundles become massed as a single bundle is not to be positively stated at this time. The individual bundles can hardly be imagined as moving together through the cytoplasm. It is probable that some of the several bundles as seen in figure 11, plate 3, are lost and absorbed, but it is not probable that all but one are so removed. The central bundle in figure 7, plate 2, looks large enough to be composed of several, such as are seen in figure 11, plate 3. The best explanation is that several remaining bundles are moved toward each other by growth currents in the cytoplasm, or by the absorption of material which lies between them. At the same time, of course, the peripheral cytoplasm is growing in mass and all nuclei tend to remain in this external layer. We shall see later that a very few nuclei are left behind in the central fibrous mass in some electroplaxes.

In figure 7, plate 2, we see, plainly and indubitably, the first form of the electroplax as found in older fishes.

The connective tissues which surround the electroplax are becoming more decided in figure 7, plate 2. Also blood-vessels and pigment-cells are oftener seen as in this drawing. One muscle cell, with its myofibrils clotted into several irregular masses and its nucleus in an advanced stage of disintegration, is seen just between the lower end of the pigment-cell and the electroplax.

It will be well at this point to examine some of the longitudinal sections of these early stages, in order to make clear several points which can not be so well studied in the transverse views.

Figure 12, plate 4, is a low magnification picture (× 140) of 6 segments of the caudal part of the body at region *D* in this 12-day embryo. Only the dorsal part of the body is shown, where a fortunate slant of the section has permitted the knife to pass through both the dorsal and the upper median spindles at the same time. The drawing is an accurate projection from three different sections, so that all parts of each spindle might be shown, as well as their relations to the rest of the myotomes. Bracket No. 1 (zone 1) in this figure indicates the inner tips of the ventral halves of the myotomes. Zone 2 shows a mass of connective tissue and nerve which runs between these two halves (compare fig. 5, plate 2, *Conn T.*). Zone 3 embraces the inner tips of the dorsal myotomes where they lie between the upper median spindle and the horizontal connective-tissue septum. It will be noticed that this set of muscle fibers appears to be very short, much shorter than the connective-tissue regions between them (not filled out in this drawing). The reason can be seen at once when these same muscle cells are examined under the high power (fig. 15, plate 5, bracket 3; and fig. 6, plate 2), where it is apparent that they are degenerating fibers. Parts of four of these fibers are shown in figure 15, plate 5, and that one nearest the electric tissue which is represented in figure 15, plate 5 (brackets 4 and 4_1), is the farthest gone, having lost its myofibrils altogether. The nuclei are also distorted and, besides the single large nucleolus, are noticeably empty of any chromatic matter or even of linin (notice the nuclei of healthy muscle and electric tissue). The next fibers to the left in this figure serve well to show how the degeneration takes place from the ends, where the cytoplasm is gathered in heavy lumps which stain light and yellowish with eosin. In fact, there is a singular similarity between the process of degeneration of these muscle cells and the transformation of the others into the electric tissue. Above, in zone 3, is a rounded cell which I take to be a muscle cell in a final stage of dissolution. It contains large granules of a chromatic substance, which appear to be the remains of myofibrils. Figure 6, plate 2, also represents three fibers from this same region in another myotome and shows three stages of the degeneration of the muscle. Two of them indicate that the degeneration begins in the middle of the fiber.

Zones 4 and 5 in figure 15, plate 5, as well as in figure 12, plate 4, show the young electric tissue of the upper median spindle. Let us first consider this tissue in figure 12, plate 4, where we can get a larger, low-powered view of it. In the first place, the most noticeable feature of the electric tissue here is that it shows scarcely any signs of a division into the original myotomes. A trace of this is seen in zone 4, but in a careful and systematic search through a number of the spindles of this stage very few instances could be noted. Even the myofibril bundles of successive myotomes seem to have united, and these bundles were most carefully examined under a Zeiss 2 mm. 1.40 ap. lens. I have no doubt that each spindle does constitute a continuous reticulum of muscle cells at this period. Laterally, the various muscle cells are not continuously united, but, as is shown in figure 15, plate 5, they are united at a number of points much in the way that some heart-muscle fibers are.

The myofibrils are best seen, of course, in these longitudinal sections, and it can be seen that they are typical. The transverse striation is as perfect as in the functional muscle, but owing to the slighter fibrils they are not quite so dark. In the upper median spindle of figure 15, plate 5, some points were found where this striation was slightly weakened. Cases of relaxation and semi-contraction uniformly characteristic of the normal muscle prevailed. The distances between the bands, as well as the length of the anisotropic parts, was the same as in ordinary muscle and remains as long as striation can be seen. This same fact does not hold true in *Raja* or in *Astroscopus*, where it is much shortened. It does hold, however, in *Mormyrus*, which is closely related to *Gymnarchus*.

The longitudinal sections, shown in figure 12, plate 4, and figure 15, plate 5, correspond to the spindles before it has become possible to see that they have segmentally divided into electroplaxes and before a central core of fibrous material has become definitely differentiated from a superficial layer of cytoplasm containing all of, or nearly all of, the nuclei.

We will now advance toward the head of this same specimen, to find material in a more advanced stage of development, in order to study the segmentation of the embryonic spindle into its individual electroplaxes (this segmentation has just been described as absent in fig. 12, plate 4), and to study further the differentiation of the inner fibrous core from the outer layer, and lastly to see how the myofibrils lose their transverse striation (muscle striation). The reader will remember that at this stage the anterior electroplaxes are in advance, developmentally, of the posterior ones. Figure 13, plate 4, shows four vertebral segments, taken from the region *C* of this same embryo, under low magnification. Bracket 1 embraces the zone of the epithelium. No connective tissue is shown. Between the muscle-zones 2 and 4 lies the zone of the long, narrow, embryonic electric spindle, marked by bracket 3. It is the lower median spindle which is shown, one which is always in advance of all the others in development. In this figure it becomes quite plain that, while the muscle tissue is arranged segmentally to correspond to the vertebra, the electric organ is not. In this case the electric spindle is separated transversely by connective tissue into three segments, instead of four. Nor is this proportion always maintained among the several electric spindles themselves.

In some cases, as will be seen later, a single electroplax corresponds to as many as five vertebræ. Even the electroplaxes do not correspond with each other. A subsequent figure will also demonstrate this (fig. 23, plate 9). Just what factors do determine the length of the electroplax I am unable to say. A closer study of the nerve distribution and blood-supply may throw some light on the matter.

The transverse sections of the stages represented in figure 13, plate 4, do not show anything of interest over and above what was discussed in connection with the conditions seen in figures 9, 10, and 11, plate 3, and figure 7, plate 2. We will, therefore, advance toward the head one step further in this embryo and examine a longitudinal section from the region marked *B* in text-figure 6. Here (fig. 17, plate 6) the electric tissue is seen in what may be considered as its maximum development in this 12-day embryo. The electroplaxes are distinct from each other and have grown considerably in size. The one selected for illustration is from a lower median spindle and shows an actual length of 0.9 mm. It appears with smooth, well-defined boundaries and good separation of core and outer layer. The myofibrils are shown in the core as several closely associated bundles, only to be distinguished from one another by a slight curving and twisting in their course. This would not be visible in a transverse section at most levels in the electroplax, and it gives strength to the view, expressed before, that the fibril bundles of a number of young muscle cells combine to form the single fibrous core of each electroplax. At the levels examined it would appear that a group of from 4 to 7 cells from each myotome is concerned in the formation of any electroplax and that these same groups of from 2 or 3 myotomes are likewise united end to end, thus making it possible that from 12 to 21 muscle cells are united to form each electroplax.

The beginning of the loss of transverse striation is quite visible in figure 17, plate 6. This striation merely fades or loses its staining power, beginning at several points, but usually at the middle of the electroplax. The ends retain the staining power of the *M* stripes longest.

The multiplication of nuclei must be spoken of at this point. It is well known that the nuclei of future muscle tissue divide by mitosis with a consequent and subsequent division of the cell-body as long as the cells are in the young myoblast stage. As soon as muscle differentiation begins to take place, the mitotic division of nuclei is changed to an amitotic type of division and the cell-body ceases to divide and begins to lay down myofibrils. Some few observations against this view are recorded, but it seems to hold in *Gymnarchus.*

In figure 15, plate 5, one can readily see many cases of amitotic nucleus division. The few cases of mitosis visible in the figure are of connective-tissue cells. Such cells, it is known, always divide by mitosis during their whole existence. As the electroplax grows, more nuclei are needed, and they are supplied by the amitotic divisions above mentioned. Just how long this process keeps up is not known, but it is probably mostly done before the first 15 or 20 days of development are passed. It is evidently going on fast in the 12-day embryo, and it is possibly finished in the 42-day stage. The nuclei are typical muscle nuclei in appearance and are not to be distinguished from the nuclei of real and active muscle in the same preparations, except, perhaps, they are slightly larger and have a somewhat heavier chromatic content (larger nucleolus and chromatic granules). This does not hold, of course, for the degenerating nuclei, whose differences have already been described. Connective-tissue nuclei can at once be distinguished by their delicate outline and small chromatin content, which is distributed as very small granules.

STUDIES OF A LARVA FORTY-TWO DAYS OLD.

In order to continue tracing this history of the development and growth of the electric tissue, we will now be obliged to pass to an embryo of some considerable size and one in which the oldest tissues are almost the same, for an understanding of the adult structure as those of a fully grown fish would be. Text-figure 7 gives an outline of this specimen, which was about 63 mm. long and whose tail part was cut off and divided and sectioned as follows: Beginning at about the level of the anus, a portion composed of 6 vertebral segments was sectioned in a vertical and longitudinal direction (region A); then passing caudad, the next 2 segments were cut transversely (region A_1); then the next 12 were cut longitudinally and horizontally (region C); then the next 6 were cut transversely (region C_1); then 13 others were cut longitudinally and vertically (region D); then 7 more were cut transversely (region D_1); then the next 14 were cut longitudinally and approximately vertically (region E), while the tip of the tail, composed of some 12 or more segments, was sectioned transversely (region E_1).

In the embryo of 12 days the youngest developmental stages of the electric tissue were found in the most distal portion of the tail, which at that time had but recently been extended by growth from the body and was still in process of extension. The oldest and consequently the most-developed electroplaxes were to be found in the anterior or cephalic end of the spindles.

In the present embryo, or larva, of 42 days, the conditions are reversed, and the electroplaxes in the extremity of the tail have passed those farther up in the body in their differentiation, and have reached a much greater and more complete development. Accordingly we will select for study one of the most anterior and least developed examples and compare it with that electroplax last studied, which is represented by figure 17, plate 6.

Figure 18, plate 6, is drawn from one of the ventral electroplaxes of the region C, as shown in text-figure 7. This position makes it fairly well forward in the spindle, although portion B would have shown slightly younger stages.

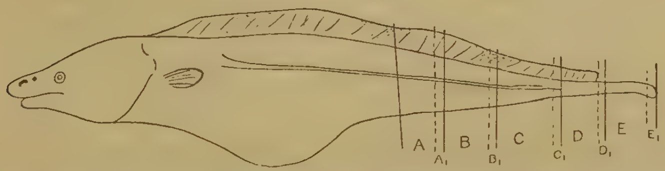

FIG. 7.—Diagram of body of an embryo, or larva, of *Gymnarchus*, about 42 days old. Lines and letters indicate regions studied. For explanations see text. (Copied from the same source as text-fig. 5.) × about 1 and 1.5.

The fibril core will first attract our attention. The first noticeable feature is that this core is growing in mass and volume all through the electroplax, but also far faster in its center than at either end. Throughout its course it has assumed a unified appearance which shows no trace of the several fibril bundles which have gone to compose it. In the narrower ends the mass is straightest and its component fibers appear most parallel, being but slightly wavy. As we follow them from either end towards the middle it can be seen that their course becomes more and more wave-like, until, in the middle, they have been thrown into decided folds. Their actual course can be somewhat better followed with thicker sections and deep eosin staining than with preparations of the usual kind.

As to the fibrils themselves, they have lost the transverse striation altogether. By this I mean that it is no longer stainable. It seems that, in the straight ends, with a good immersion lens one can see traces of this striation, left as a thickening of the fibrils, at points that may represent the previous position of the anisotropic *M* spindles.

Whether the increase in the mass of the fibrillar core, which goes on as the electroplax grows, is due to the thickening of the individual fibrils or to the laying down of more and new fibrils or to the deposit of an interfibrillar substance between them, was not decided. The first two conditions seemed the most probable, because of the apparent absence of much interfibrillar substance in the oldest and largest electroplaxes.

The cytoplasm of the outer layer begins to be of interest at this stage. Its most particular point of interest lies in the fact that it is decidedly different in structure at its anterior end from its posterior end. This is shown in its staining capacity as well as in its actual structure. In the specimens stained in iron hæmatoxylin and eosin the cytoplasm at the posterior end stains deeper than that at the anterior end, with both dyes. It can also be seen to be granular in structure—a sort of general granulation with a few larger granules of a substance which stains somewhat like chromatin. In particular, its cytoplasm is darker than the fibrillar core at this posterior end.

As the cytoplasmic layer is examined in an anterior direction, it is seen to stain lighter and at the same time to contain an occasional vacuole. This condition increases until, at the anterior end, about one-fifth of the length of the entire structure is covered with a cytoplasm which is so much vacuolated that it appears as a delicate reticulum in the meshes of which the nuclei lie. The fibril core extends out to the end or almost to the end, and it can be seen that it is darker in color and denser than the cytoplasm covering it. Some of the finer, granular material is found out as far as the reticulated tip.

A new element of interest begins to become apparent in this stage, and that is the point of attachment of the nerve. The strong medullated fibers come from the cord, pass through the spinal ganglion, and enter the connective-tissue "tube" of the spindle at the level of the posterior third of each electroplax. The fibers wind and turn considerably, in this vicinity, and are finally applied to the surface of the electroplax over an area which may be described in this specimen as its second sixth part from the posterior end. Thus the extreme posterior end does not receive any nerve-endings at this period.

The cytoplasm shows a number of indentations where the nerve is applied and the axis-cylinders of the nerve can be traced into these spaces, which they apparently fill with a club-shaped nerve-ending. This ending can not be satisfactorily described until some of the special nerve methods can be used to elucidate it.

Another and more simple step in development is indicated in figure 19, plate 6, which was taken from an electroplax in a dorsal spindle of this embryo at the region *E* (see text-fig. 7).

Two points of interest will be spoken of in connection with this stage. The shape has changed as follows: The middle part has both actually and comparatively widened over the breadth shown by the middle part of the electroplax seen in figure 18, plate 6, and this increased width is due to a broadening of the fibrous core alone, the outer nucleated layer remaining the same.

Accompanying this widening is also an actual shortening of the structure. Thus, part of the increased bulk of the middle is due to an absorption of the two ends. Apparently the anterior end suffers the greater amount of absorption, for it is usually shorter and thinner than the posterior. In this connection we must remember the previous vacuolization of this anterior end as seen in figure 18, plate 6. It would appear that the vacuolization was part of an absorption, or rather of an atrophic process. Considerable traces of it remain in figure 19, plate 6, and it is also still noticeable that the anterior end does not stain deeply. The posterior end retains its length and vigor to a greater degree. It seems to become considerably narrowed, however.

The form of the electroplax also begins to show a marked change through the development of spurs, branches, or papillæ which begin to protrude from both its ends, particularly from its posterior end. Some indication of this was visible already in figure 18, plate 6, as small lumps or "shoulders" that marked the organ roughly into three parts—anterior, middle, and posterior thirds. These growing spurs begin to give the middle third a distinct truncate or cylindrical form. The nerve fibers also attach themselves to the sides of the growing papillæ, particularly to their bases. The papillæ show an inclination to grow out of the sides of the posterior third.

We will now pass to the last and oldest stage of the electroplax which can be found in the embryos which Budgett collected in Africa. This is found in the *E* region of the 42-day embryo or larva of *Gymnarchus* and is represented in longitudinal sections under a low magnification by figure 23, plate 9, which shows parts, or the whole, of about 11 electroplaxes lying in place in the ventral, lower middle, and upper middle electric spindles of this region. Figure 21, plate 8, also shows a transverse section just cephalad of this point, in the *D* region, where cross-sections of the 8 electric spindles reveal partial or complete transverse sections of 7 electroplaxes, but only the electric connective tissue which fills the tube at this point, in the eighth.

Figure 20, plate 7, represents a longitudinal section of one of the electroplaxes shown in figure 23, plate 9, and will serve as a basis for our last study of this series.

The form has continued to follow the development which was indicated in figure 19, plate 6. The total length of the structure has shortened somewhat more, while its width in the middle part has increased twofold. These statements are the result of averages of about 15 measurements. The papillæ on the posterior surface have increased in length and some of them have begun to rival the posterior portion of the organ in length. We have no suitable figures of the adult electroplax, but from Fritsch's (3) descriptions, and from the tendencies shown by these embryos, it would seem that, as Ewart describes in *Raja*, the original posterior portion of the electroplax shortens and the new papillæ lengthen until they all form approximately similar structures. This can only be completely studied when we have secured suitable sections of the grown fish.

The development of papillæ is noticeably weak on the anterior surface. The figure does not show as many as some of the electroplaxes in figure 23, plate 9, but it is a fair illustration. Neither does it show well the usual condition of the main anterior process of the cell at this time, which can be better seen, in some electroplaxes of figure 23, plate 9, to be still in evidence and of considerable length, but of very weak development. This anterior process shows no trace at this age of the general vacuolization of its cytoplasm which we saw in an earlier stage, and the fibrils extend as a very thin and uncertain core through its length.

The fibrillar mass which forms the core of the electroplax has now assumed what appears to be its permament condition. The fibrils are very fine and, after having passed straight down through the posterior process, are thrown into flat waves by the process of packing them into the shortening and widening middle part which now constitutes the principal bulk of the structure. This causes the larger part of the fibrils to lie nearly at right angles to the longitudinal axis of the organ, and this appearance was first taken by the writer, who examined the oldest embryo first, to be a trace of the transverse striation of muscle from which the organ is formed. We now know it to be the myofibrils, lying at right angles to their original course. No conclusion was arrived at, in this stage, concerning the growth of this mass, as to whether the number of fibrils was increased, or whether the larger size was due to a thickening of the original fibers, or to the deposit of interfibrillar substance. The fibrils seemed to be as fine if not finer than in the earlier stages; certainly they are much finer than functional myofibrils. Since muscle tissue increases the number of its myofibrils long past this age, I see but little reason why this modified muscle should not also do so.

A closer study of the cytoplasm and nuclei of the peripheral layer was next undertaken in this oldest stage. Beginning on the posterior surface and on the papillæ, we find the layer thickest here and composed of at least three distinguishable materials. One was a dense material which was reticular in structure and stained with chromatic stains deeper than almost any other pure cytoplasm that I know of. This material was one constituent, while the other substance composing the general field at this point was a far lighter staining material which was homogeneous and clear. This latter seemed to bear the same relation to the denser material that the "nuclear sap" does to a linin alveolum or reticulum in the nucleus. Like the linin reticulum, this denser material was more refractive and took more of the chromatic as well as more of the acid counterstains than the homogeneous material did. I shall adopt Schneider's (31) name of "Linom" for the denser substance and his name of "Hyalom" for the clearer and more homogeneous material. It seems probable from the works of Bütscheli (9), Rhumbler (27), Hardy (35), Wilson (34), Andrews (1), Strasburger (32), and others that these structures of cytoplasm, as seen under the microscope in fixed material, do not represent the exact condition as it exists in life. After reading the pages of my recent work on the electric-motor cells of *Torpedo*, one can more easily see that the cytoplasmic linom is a structure which depends upon the fixative used as one factor and upon the chemical and physical peculiarities and the contents of this plasma at the time that the fixative is applied, as another set of factors. Its reticular or alveolar arrangement can most certainly be immensely varied, and all these artificial conditions must be very much different from that which obtains in life. Since I have only a few fixed specimens to discuss, over whose earlier preparation I had no control, I shall not try to solve the question of what the structure was during life, but will merely describe the present specimen as it appears.

The linom of the cytoplasmic layer on the posterior end is very fine and can only be seen with the best lenses and under the best conditions. This holds particularly for the outer portion of the layer, for as we examine the inner portion the reticulum grows larger meshed until, at its point of contact with the fibrillar core, the meshes are quite easily seen.

The same is true as we examine this layer in a more anterior position. Here all the meshes are proportionately larger, until in the layer, as found covering the extreme anterior surface, the meshes of the linom are visible with ordinary high powers. I do not refer to the vacuoles which are found at various points, for there is a great difference between the largest meshes and the vacuoles, although the vacuole may be derived from, or originate in, an overgrown mesh. The meshes always contain the hyalom, while the vacuoles do not. My definition of a vacuole in this case will be a space in the linom into which the hyalom does not extend. Also, the rounded outline of a vacuole shows a surface tension between its content and the cytoplasm, which does not seem to exist between a mesh of the linom and its contained hyalom. These vacuoles appear to have contained a soluble substance during life, and the hyalom is not soluble in fluids used to prepare the specimen. Such vacuoles are found at various points in the cytoplasmic layer, most often at its outer edge, while the large meshes appear at the inner edge and around the nuclei. The vacuoles become so large and numerous at the anterior end of a stage such as figure 18, plate 6, that the cytoplasm looks like foam containing the nuclei and surrounding the unchanged fibrillar core.

In addition to this structural basis of linom and hyalom, another very prominent content of the cytoplasm is a series of granules of some material that is denser and somewhat more stainable than either of the others. These granules are very numerous and fine at the periphery, where they cause the outer part of the layer to stain deepest with eosin, and they lie in the finer-meshed linom, and grow larger and fewer as one examines the inner parts of the layer. They are of a slightly more refractive quality than the linom and stain deeper with chromatic dyes the larger they get. The largest also have some color of their own, a slight golden-brownish color, somewhat like that of pigment granules. While the smallest granules seem to lie in or attached to the strands of linom, the larger seem to lie in its meshes in the hyalom. They remind the observer of the granules found in certain other cells, particularly in the electric-motor cells of *Torpedo*, as well as in other nerve-cells. In figure 16, plate 5, these finest granules are seen in the outer part of the layer stained with eosin, while the inner part does not show the larger ones that lie there.

The larger granules are prone to gather about the nuclei and about the inner part of the electric layer, as seen in figure 22, plate 8, where they took the iron hæmatoxylin stain well. The larger, chromatic-staining granules are also found, in groups or singly, in many parts of the fibrillar core. While the larger ones found in the core, around the nuclei, and at the inner edge of the cytoplasmic layer seem to stand in sharp contrast to the more numerous and finer ones found in the outer part of the cytoplasm, one can trace steps between the two kinds.

The writer believes these granules to be the secreted or prepared nitrogenous material used by katabolic processes to produce electricity, either directly or indirectly. They must be, physiologically, the same granules described by Ballowitz (5) in the cytoplasmic layer of the electroplax of *Raja*, and by Schlichter (30) in the cytoplasmic regions on both surfaces of the electroplax of *Mormyrus*. In this latter they were very large and not easily demonstrated. The writer has seen some indication of them in *Astroscopus* (12), but they would seem to be noticeably absent in other forms, as *Torpedo* (although they have been figured here by Fritsch (20)), and in *Gymnotus* (3) and *Malopterurus* (6), where such granules as Ballowitz has described or figured would seem to be inadequate in size and number for so heavy a duty. It will be noted in the above list that the weak-electric fish seem to have these cytoplasmic granules best developed, while the strong-electric fish show least of them. It is possible that these latter possess them, or their equivalent, in a more fluid and less visible form.

The cytoplasmic layer is marked off from the fibrillar core by a very definite and sharp line. It can not be said that a definite membrane exists here, although one may exist. The boundary between the outer layer and the core is not an even or a straight one, but shows a wandering course, especially at the two ends. At some points strands of the linom seem to branch into the core (fig. 16, plate 5). At the anterior end in particular it shows many extensive and complicated invaginations of its granule-containing substance into the fibrillar core. Certain small portions of it also have been permanently left in the main body of the core, usually near its anterior end. These inclusions (fig. 20, plate 7) may or may not include the nuclei. They always contain some of the largest of the granules.

The nuclei do not show any internal peculiarities which would distinguish them as electric nuclei from some of the other tissue nuclei, particularly from muscle nuclei, which they much resemble. They have a large chromatic content and a particularly large plasmosome which stains deeply. They can be sharply distinguished from some other nuclei, as connective-tissue nuclei for instance, where the more delicate outline and different chromatic pattern is discernible at a glance.

Each nucleus shows some sign of a surrounding differentiated layer of cytoplasm. This consists of larger granules and a zone in which the hyloplasm seems to be in greater proportion than elsewhere. At different places in a preparation one may see more or less of a contraction zone around the nuclei. While this may be a physiological condition, it is more probably an artifact due to the fixing or hardening.

One interesting condition is to be seen in most of the few nuclei which become detached from the outer layer and included with some small portion of the outer cytoplasm in the fibrillar core. These nuclei probably become so placed during a very early stage, and the further they are separated from the layer to which they rightly belong, the larger they grow and the more diffuse their chromatin becomes. The plasmosome diminishes in size and the whole structure looks more like a connective-tissue nucleus, except that it is very much larger. I have seen this same condition in the electroplax of *Raja*.

It was not possible to find a real electrolemma or cell-membrane covering this electroplax. A connective-tissue covering, more or less closely applied to the surface, was always present, but the fact that this covering possessed its own nuclei seems proof that it was a real connective-tissue covering and not any product of the activity of the electroplax tissue. At such points, as this connective tissue did not actually touch the electroplax, a careful examination was made to see if some actual cell-membrane did exist. Beyond the fact that the outer edge of the electric layer was sharply defined and that its surface was rounded and even as if some membrane was present, no real membrane could be demonstrated, either by its refractive properties or by its color.

INNERVATION.

A general survey of the innervation is desirable, as too little exact topographical work has been done on those fishes in which the electric-motor centers are thinly distributed in character over large areas of the cord, as, for instance, in *Gymnotus*, *Raja*, and the mormyrids. Regions *D* and *E* were selected in the 42-day-old embryo as the most favorable parts to study. The work was not as exact as it could be if the investigator had had plenty of live material, especially adult material, on which to use some of the well-known neurological methods. But even in this embryo, which was well fixed in sublimate-acetic, much could be accurately made out, and it is hoped, too, that the description will prove suggestive.

The motor cells were first looked for in the spinal cord, especially that region which was posterior and in the neighborhood of the electric organs. Assuming for the present, as a law, that electric-motor cells are larger than muscle-motor cells which innervate an equal weight of muscle, it was very easy to find groups of large, heavy nerve-cells scattered through the substance of the spinal cord from near the anterior beginning of the spindles to the very last point in the tail to which they extend.

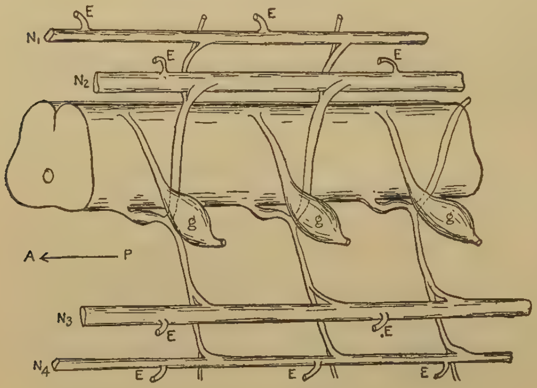

Fig. 8.—Side view of reconstruction (semi-diagrammatic) of spinal cord and motor electric nerves of a larval *Gymnarchus* 42 days old. Arrow indicates anterior and posterior directions. N_1, N_2, N_3, and N_4 are the four electric nerves formed by branches from motor roots of spinal nerves. *E* indicates small branches from electric nerves that innervate posterior surfaces of electroplaxes. *g* marks spinal ganglion, whose afferent nerves have been cut off.

These cells were situated just above the central canal and from 1 to 4, or even 5, could be seen in every transverse section in most of this length (fig. 14, plate 4, *A*, *B*, and *C*). They did not appear to be divided into two symmetrical groups, but rather to lie in one median group. On the one hand, their dorsal position might appear to be evidence that they were not motor-cells or that they were not to be considered as modified muscle-motor cells. On the other hand, the presence of real ventral muscle-motor cells (fig. 14, plate 4, *D* and *E*) in the whole length of the cord, the correspondence of the cells under discussion with the position of the electric spindles, and the fact that Fritsch describes similarly placed cells in the same position in mormyrids as the motor-nerve cells of the electric organ, all seemed to constitute very strong indirect evidence that these were the nerve cells which innervated the posterior ends of the electroplaxes.

In addition, the writer was able to trace, in a number of cases, the course of the neuraxons through the cord, out into the ventral roots of the nerves, and finally into special bundles of nerve fibers that undoubtedly innervated the electric tissue. No one nerve process was actually traced for the whole distance unbroken, but the various parts and regions were so pieced together that the course was well established and corresponds in many ways with Fritsch's observations on *Mormyrus*. Text-figure 9 shows a semidiagrammatic cross-section of these courses as seen from in front, while text-figure 8 shows the same thing sketched in relief from the side. This topography will presently be described.

The electric-motor cells at this age must of course be considered as still very young, and descriptions from the adult are desirable. We will begin by examining the 9-day-old embryo once more to see if they have started. In this cord (fig. 8, plate 2) there are but very faint traces of any nerve-cell development, and some neuroblast mitosis is still going on. In the position to be later occupied by electric cells a little enlargement of nucleus and cell-body is visible. One cell here is of great interest, and that is one of the now well-known "Hinterzellen" or giant cells, first described by J. Beard (**37**), Rohon (**29**), Studnitzka (**33**), and others, in the embryos of *Salmo* and *Raja*, and later by Fritsch (**21**), as a different sort of cell, in the adult *Lophius*. Still later, such cells were described by the writer (**36**) in the embryos and adults of various Pleuronectidæ and in *Pterophryne*, where he showed it to be the same cell in both embryo and adult, and described the relations of anterior and posterior branches of the neuraxon. From its size, shape, and position in the present specimen, it seems that this dorsal cell might be, in some way, related to the electric-motor cells, but that question is easily settled when one examines the 12-day embryo and finds that all of the dorsal cells have effectually disappeared before the electric-motor cells begin to differentiate. Besides, the well-known fiber courses of the dorsal cells as worked out by Fritsch in *Lophius*, the writer in *Pterophryne*, Harrison in *Salmo*, and Johnson in *Catostomus* are sufficient proof that the two have nothing in common.

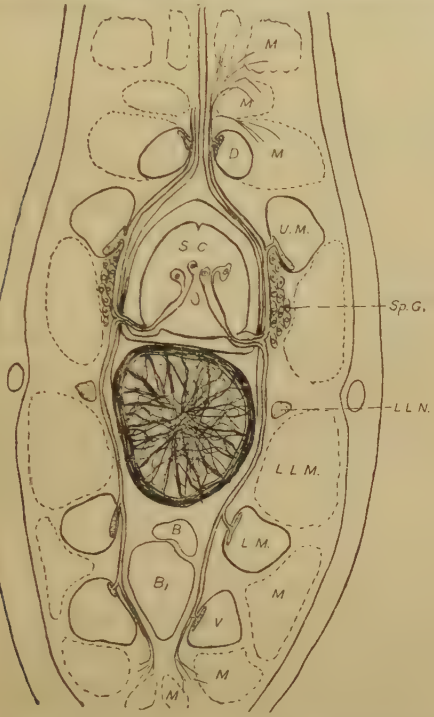

FIG. 9.—Diagram to show position and relations of nerve elements to electric spindles. *S.C.*, spinal cord, showing central canal and four motor electric nerve-cells. Processes from these cells pass out through ventral roots, and distribution of these roots to electric spindles and muscle-masses is indicated. *M*, some of the muscle-masses. *D*, *U.M.*, *L.M.*, and *V* show electric spindles on one side. *Sp.G.*, spinal ganglion; *L.L.N.*, lateral line nerve.

The 42-day larva shows the best development of these electric-motor-nerve cells until an older fish can be described. Here we find a heavy, rounded, cytoplasmic body of about 40 microns in diameter (fig. 14, plate 4, *A*, *B*, and *C*). Its outline is usually pear-shaped, with the axis-cylinder process given off at the pointed end. Dendrites are not visible in the specimen at hand. While most of these cells are of the same size, a few are noticeably smaller.

The nucleus is large, round, and placed nearly always in a very eccentric position. This position is most frequently on the side away from the neurite, but sometimes it may be very close to the neurite. Its diameter is about 16 to 19 microns in the largest cells of this age, and its outline is hard and round. The nucleolus consists of a single, or rarely double, plasmosome, which is a very little less than 5 microns in diameter, but which, when double or multiple, is of proportionally smaller size. As I have shown in the nucleus of the electric cells of several torpedoes, when a plasmosome is multiple its several parts, collectively, are larger in mass than is a single normal or usual plasmosome.

Of course, the nucleus was carefully examined as to any possible orientation of its nuclei, particularly the plasmosome, with reference to gravity or to the electric current or to the axis of the cell. Nothing of this sort could be found, although this does not preclude such a condition in the grown fish. It is known that in *Torpedo*, where such an orientation does exist, this same orientation is not found in the embryonic or larval stages.

From these cells thin, delicate axis-cylinder processes were traced down, as has been described, and into the ventral roots of the spinal nerves in a sufficient number of cases to assure the observer that they all ran in this direction. The process was thinnest shortly after leaving the cell, and became thicker as it approached the nerve-root. When it once entered the root it again became very thin, although now invested with a connective tissue and a medullary sheath. A large number of connective-tissue nuclei, nerve-sheath nuclei, and some unknown elements cause the motor-root of the nerve to swell to some size just after leaving the cord. It decreases again in size before it enters the foramen and leaves the vertebral canal in company with the much smaller dorsal root.

Just outside the canal the dorsal root ends in the spinal ganglion, and the motor-root traverses the inner side of the ganglion, from which it emerges on the lower edge, and at once divides into a dorsal and a ventral branch.

The ventral branch passes backward and downward (see text-figs. 8 and 9) to a point at a level with the lower middle spindle, and here it gives off a considerable group of fibers which pass caudad just inside of the spindle capsule. These fibers are joined by similar groups from others of the spinal nerves and the whole mass forms the lower middle electric nerve (text-fig. 8, N_3). At the posterior level of each electroplax this nerve gives off a few fibers (text-fig. 8, *E*) which branch out and innervate the posterior surface of this electroplax.

The remainder of the ventral branch passes farther down and again gives off a branch which goes to form part of the ventral electric nerve (text-fig. 8, N_4). This latter sends off little branches to furnish the posterior surfaces of the ventral electroplaxes with motor-fibers (text-fig. 8, *E*).

Going back to the anterior root, we find that its dorsal branch leads directly upward and, passing between the spinal ganglion and neural arch, slopes gently backward to give off a large branch which becomes a part of the upper median electric nerve (text-fig. 8, N_2). Its remaining fibers reach upward and furnish the dorsal spindle with a part of the fibers that form its dorsal electric nerve (text-fig. 8, N_1).

Of course, there are muscle-motor elements in both these branches of the anterior nerve-root, the number depending upon the position, backward or forward, at which we examine the arrangement. Two examples of the motor-nerve cells of the muscle-tissue from the cord in region *D* are shown in figure 14, plate 4, *D* and *E*. At the level of the anterior parts of the electric organ, when there are large quantities of muscle and the electroplaxes are very small, the muscle branches are large, particularly the dorsal branches, which have to supply the muscle-bundles for the large dorsal fin. In the posterior region of the tail, on the other hand, the muscle is almost entirely absent and the muscle-branches of the anterior roots are not even easily seen.

The eight (four on each side) longitudinal electric nerves are interesting in that they form a morphological buffer between the conflicting segmentation of the nervous, skeletal, and muscular systems, and the independent segmentation of the electric system. Were the electric segmentation to correspond with that of the others, we should not find these nerves in this recognizable form. In fact, in the anterior part of the electric organ we do not find them as continuous nerves, in many places, for more than two or three neuromeres at a time. And even posteriorly where they do form continuous nerves, the fibers that enter them from any given spinal nerve do not pass very far back in them before leaving to innervate one of the electroplaxes. Each nerve cell probably lies but a very short distance in front of the electroplax which it supplies. This was decided upon by plotting the relative positions of all motor-electric cells and all electroplaxes in the larger part of the organ.

While doing this it was also determined how many nerve-cells sent their nerve-processes to each electroplax. Thus, in region *E* of the 42-day-old larva there were easily counted 414 of the electric-motor cells, while in the same part there were 81 electroplaxes. This makes it quite sure that, on an average, about 5 of the nerve-cells were used for each electroplax. It was attempted to count the nerve-fibers as they left the electric nerve to branch out over the electroplax, but the elements were too small and the fixation not just what was needed to do this. It could be easily done in a grown specimen.

I shall now take up the structure of the nerve-fibers as they leave the electric nerves to pass to the electroplaxes, assuming that they have no peculiarities of interest during their course through the nerve-tracts between the cord and the electric chambers. (The electric chambers are not divided as in *Mormyrus* by a transverse septum of heavy, opaque, white connective tissue; this can be explained by the fact of their secondary segmentation.)

As the few fibers destined for any particular electroplax first leave the electric nerve they are directed caudad and are very small in diameter and invested with a fine connective-tissue sheath. This covering is probably medullated in the grown fish. They quickly turn in a gentle curve whose diameter is about half that of the spindle and pass forward through the electric connective tissue to the posterior surface of the electroplax. At the beginning of this curve, or just after leaving the electric nerve, the axis cylinder or neuraxon enlarges to a considerable diameter and becomes very wavy and irregular in diameter. Its course is no longer straight, and it is not possible to find a section of any considerable part of its length, except in some very few cases. Its sheath of connective tissue is loose fitting and the inner side shows a loose reticulum of fine fibrils and plates that form a weak connection between the axon and sheath.

It is at once apparent in the maze of fibers which approach the electroplax that the few neuraxones which first entered the compartment are now many in number. Still, it is difficult to see where they branch in the thin sections, owing to their sinuous and irregular courses and to the large numbers of transverse and oblique sections present. In several places this branching was seen, however, and recognized as the same multiple branching which has often been described in the terminal part of nerve-paths and, in particular, in the same comparative region of the electric tissue of *Raja* by Ballowitz (5) and Retzius (26), in *Torpedo* by several authors, and especially in *Malopterurus* by Ballowitz (4), who refers in this article to many other cases. The writer has also seen and figured it in the electric tissue of *Astroscopus* in a paper soon to appear. In the present case, one section, as can be seen in figure 16, plate 5, exhibits two cases of this branching, one of them showing a single fiber dividing into at least three or four branches; also, figure 25, plate 9, in which there is to be seen a well-defined single fiber dividing into two branches just before they end in two club-shaped nerve-endings in the electric layer of the posterior surface of an electroplax. The abundant nodes of Ranvier, described by Schlichter (30) in the case of *Mormyrus*, were not observable here, probably on account of the lack of osmic-acid fixation. I have no doubt that they are present and they must be present at the points at which the fibers divide.

As has already been stated, the fibers now approach and end on the posterior surface of the electroplax, as well as on the lower sides of some of the papillæ that arise from it. The mode of ending is not difficult to see, apparently, although this much studied and controverted question should not be approached lightly, especially where the material is merely a sublimate-acetic fixation stained with iron-hæmatoxylin and eosin.

The nerve-process, carrying its connective-tissue sheath until it actually reaches the surface of the electroplax, ends in a blunt and somewhat thickened knob which is embedded in a hollow or invagination in the substance of the posterior cytoplasmic or electric layer of that structure (see figs. 18 and 19, plate 6; also fig. 16, plate 5; also figs. 24 and 25, plate 9). This knob may be quite elongate in form and in some cases appears to be branching. The nerve fiber becomes very narrow and apparently dense just before entering the cavity, but it quickly broadens out, to as much as or more than its previous width, to fill the cytoplasmic cavity of the electroplax. Its substance becomes very light-staining, more so than any other part of its length is, and this light-staining quality is most apparent at its extreme distal end in the cavity. The nerve-fibrils, faintly visible in the outer courses of the axon and rather more so in the denser neck just before entering the cavity, can be seen in the light-colored club-shaped ending to be running in an irregular reticulum instead of in their previously almost straight and parallel manner. They could not be traced into the protoplasmic bridges between the club-shaped nerve-ending and the surface of the protoplasmic cavity in which it lies. In very few instances did the nerve-substance fill the recess in the electric layer tightly, probably owing to some shrinkage in both of the tissues. The small space between the two surfaces is crossed by the numerous fine processes or strands, mentioned above, of some of the nerve-tissue remnants, probably of an original closer contact.

The question now arises as to whether the cavity or depression which the nerve-ending occupies is to be considered as an invagination of the surface of the electric layer or as a real penetration of the electroplax by the nerve. I shall consider it as the former, because the surface of the electroplax appears not to be interrupted by the opening but to continuously follow the inner edge of the cavity all the way around.

The presence of a perceptible cell-membrane or electrolemma would assist in the solution of this question, but such an organ could not be demonstrated. The edge of the cytoplasm was sharp and definite, but no membrane was visible, either by its staining properties or by refrangibility. It possibly will be found in the adult organ. Nor was it possible to see the "Stäbchen" or rodlets which have been described in other electric organs by Ballowitz (3, 5) and his pupils (30). Some striated arrangement of the granular electrochondria or granules described previously was observable, but this constituted a fibrillar secretory striation, such as is seen in the surfaces of most cells that are undergoing exchanges of any kind. Here, again, we must await examinations of the adult electroplax before we can say if such "Stäbchen" are present; also if they are homologous with the well-defined rodlets found by Ballowitz in *Torpedo* and in *Raja*, and whether they are specific structures of any importance in the production of electricity.

Returning to the relation of nerve-ending to electroplax, we have practically decided that the nerve-club is embedded in an invagination of the surface rather than in a cavity in the substance of the electric layer.

In doing this it has formed a much more intimate connection than may be seen in other places where the nerve-fibers touch the electroplax very closely, even being partly embedded in it, or running through the fundus that lies between two papillæ, where the fiber is closely pressed on three sides by the surface of the electroplax. In these fiber contacts the connective-tissue sheath persists, while in the club-shaped or heavily rounded endings the connective-tissue sheath is lost, left at the surface, and the little protoplasmic bridges, shown when slight shrinkage has taken place, testify to the intimacy of the contact. In addition a slight amount of fine, golden-colored granules surrounds the nerve-ending, lying on its surface, between it and the substance of the electroplax (figs. 24 and 25, plate 9). These granules are not found on any other part of the nerve surface.

Naturally the paper of Schlichter (30) was carefully examined to see what he had found as to the ending of the electric nerves on the related form, *Mormyrus oxyrhynchus*. He had adult material, but otherwise was no better off than the writer in the possession of material which had been treated especially for neurological study. He describes the nerve-fibers with their medullary sheaths as coming in contact with the large process of the electroplax and then suddenly ending just as they reach certain large indentations of the surface of this electroplax. He found in these indentations only a little coagulated material and some nuclei.

The writer has no doubt that the slight coagulum represents what remains of a club-shaped nerve-ending similar to that which he finds in *Gymnarchus*. The nuclei, from their position in Schlichter's picture, are evidently the nuclei of terminal connective-tissue coverings. If this idea be correct we will have a very simple but interesting form of nerve-ending, much larger in size than that found on any muscle or any other electric organ and one in which it will be, apparently, easier to study the intimate contact of nerve-substance with motor-substance from a cytological point of view than in any other form. In particular, we should try to stain these endings with the nitrate of silver and methylene blue methods devised for neuro-cytological studies. This work, however, can be undertaken only on the ground, with good laboratory facilities and with an abundance of fresh material.

The writer has published observations on some peculiar horizontal, pointed rods, or pointed threads, found imbedded in the electric layer of the electroplax of *Astroscopus*. At that time he suggested that they might be in some way homologous with or related to the "Stäbchen" because of the absence of any other well-defined "Stäbchen" in this fish. Such structures are not found in the present *Gymnarchus* larva, but they have been seen and described in *Raja*, in a paper soon to be published. Their presence in *Raja*, in addition to the "Stäbchen," proves them to be entirely different cell organs.

One word in regard to certain possibilities for the physiological study of the electric organ in *Gymnarchus*. A recent paper by Bernstein and

Tschermak and another by Bethe go to show that the electric discharge of *Torpedo* and other fishes is produced by different concentrations of sodium chloride in the electroplaxes and in the intervening electric connective tissue. Since there is a long series, theoretically, of these alternate segments of higher and lower degrees of concentration of the electrolyte, and since all the higher concentrations are presumably equal, and all the lower concentrations are equal, we would have a series of equal potentials alternately opposed to each other, and the result would be zero or else only the strength of one concentration current, in case there was one more or one less of either of the concentrations.

To obviate this difficulty a membrane has been imagined, on one surface of each electroplax, presumably the electric or nerve-ending surface (posterior surface in this case), which will be permeable only to one kind of the ions, either negative ions or positive ions, and by which the current is thus rendered integral in one direction.

Two things remain to be proved in connection with the above theory—the fact of different concentrations and the presence of such a membrane. This can be done in any fish in which it is possible to effectively separate the segments in a fresh state, so as to submit them to delicate chemical tests. In *Gymnarchus* we have a fish whose elements are larger than those in any other one of the seven electric types—large enough to be cut apart, I believe, and analyzed separately in the chemical laboratory; also large enough to submit anterior and posterior surfaces to physical tests that may show its permeability to either positive or negative ions and its impermeability to the other kind in one direction. This experimental work can most certainly be done if the proportion between the bulk of electric connective tissue and electroplax remains the same in the adult as in the larva (see fig. 23, plate 9). Fritsch shows much less of the electric connective-tissue segments in his figures of the adult organ.

Numerous other anatomical features of *Gymnarchus* have caused it to be classed with the other mormyrid fishes. This fact makes it of interest to compare its electroplax with the very different electroplax in these fishes.

That found in *Mormyrus oxyrhynchus* will serve as a type and its general plan has been well shown by Ogneff (25) and Schlichter (30). Here it is evident that a number of consecutive and entire myotomes have been converted into electroplaxes and that the middle layer of each electroplax is composed of unaltered and clearly striated myofibril bundles. The large number of these fibril bundles, and their distribution, indicate that the whole electroplax in *Mormyrus* is a syncytium composed of all or most of the cells which would otherwise have gone to make up the single myotome. In this we find an agreement with the electroplax of *Gymnarchus* which is also formed from several cells. In the one case, however, all the cells in the myotome have been used (*Mormyrus*); in the latter only those lying in eight particular localities (*Gymnarchus*). (See paper by Dahlgren (36).)

Further homology is seen in the disposition of the probably superfluous myofibrils. In both forms they are relegated to a middle position in the electroplax, while the apparently more important cytoplasm forms layers on the anterior and posterior surfaces of the structure. Also, in both, the now useless myofibril bundles are packed out of the way at right angles to the axis of the electroplax, which remains the same as the former axis of the muscle-cells that were used to form it.

The only difference lies in the fact that the striation of the fibrils is retained in the *Mormyrus* forms, while it is lost in *Gymnarchus*, the dark-staining anisotropic substance apparently dissolving away.

From what little can be predicted concerning the possible origin of the electric tissue in the other teleost forms, it is probable that the Mormyridæ (including *Gymnarchus*) are the only fish in which the electroplax is formed as a syncytium from more than one cell. In *Astroscopus*, *Electrophorus* (formerly *Gymnotus*), and *Malopterurus* the structures show every evidence of having been developed from single myoblasts with the exception of *Malopterurus*, where it is a question as to whether they are not evolved from gland-cells instead.

SUMMARY.

In conclusion it may be stated that—

(1) The electric tissues of *Gymnarchus* are developed by the differentiation of certain portions of its normal, striated muscle-tissues during an embryonic or larval period extending from the ninth day to the forty-second day of embryonic life. The critical period of this change takes place in the neighborhood of the eleventh, twelfth, to fifteenth days.

(2) The muscle fibers which go to form the electric tissue give up their usual segmentation into myotomes, and first form eight long and continuous spindles which afterward segment, each into a lesser number of masses, the electroplaxes.

(3) The myofibrils lose their transverse striation and form a large inner core for each electroplax. They lie in a wavy mass, mostly at right angles to their former course. The active cytoplasm forms an outer layer which is denser and stains deeper on the posterior than on the anterior surfaces. It contains granules.

(4) Each electroplax is made up of several muscle cells, 12 to 20 or more. This is different from the two elasmobranch fishes, in which each muscle cell forms only one electroplax.

(5) The nerve is distributed on the posterior surface and ends in blunt knobs that lie in cavities formed by the invagination of the surface of the electric layer. The current probably runs from tail toward head, as in *Mormyrus*.

(6) The development of the tissue gives a strong clew to the probable development of the electric tissues in the other mormyrid fishes.

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EXPLANATIONS OF THE PLATES.

Plate 1.

Fig. 1. Transverse section through body of 9-day-old embryo of *Gymnarchus* in region *C*. *L.M*, position of the large muscle cells that will eventually change into the lower median electric spindle; *V.F*, location of large vertebral fibers; *XXXX*, locations of electric spindles in an adult fish with reference to bony structures, blood-vessels, and spinal cord; *N*, canalis centralis; B_1 and B_2, caudal blood-vessels. × 110.

Fig. 2. Muscle-tissue in transverse section from 12-day-old embryo of *Gymnarchus* to show typical muscle cells. *A*, one of oldest fibers with two strong fibril bundles; *B*, growing muscle cell with fibrils being laid down in a ring; *C*, extreme upper edge of myotome with youngest cells at top. × 1150.

Fig. 3. Transverse section through group of larger muscle cells found at *L.M.* in fig. 1. Under greater magnification. For explanations see text. × 1150.

Fig. 4. Longitudinal vertical section of *C* region in 9-day-old embryo, ventral portion. Between marks o o are seen same parts of seven myotomes as are shown at *L.M.* in preceding figure in transverse section. Note heavy development of these cells. A line drawn between marks ⊕ ⊕ would indicate location of seven groups of vertebral fibers in next few mediad sections.

Plate 2.

Fig. 5. Transverse section of body of 12-day-old *Gymnarchus* embryo in region CC_1. *D*, dorsal electric spindle; *U.M*, upper median spindle; *L.M*, lower median spindle; *V*, ventral spindle; *Conn.T*, connective-tissue septum; *N*, canalis centralis. B_1 and B_2, caudal blood-vessels. × 110.

Fig. 6. Three degenerating muscle cells from another (3) zone in fig. 12. × 1440.

Fig. 7. Electric spindle showing first stage where electric muscle cells can be called an electroplax. Complete coalesence has taken place. Nuclei are in peripheral layer. Myofibrils are concentrated in middle to form central fibrillar core of structure. × 1150.

Fig. 8. Transverse section of upper dorsal part of spinal cord in a *Gymnarchus*, embryo 9 days old. Neuroblasts, in region that will later show electric motor-cells, show some little differentiation and growth. Near, and above, this region is one of the transitory giant ganglion cells. × 940.

Plate 3.

Fig. 9. Transverse section of left ventral spindle from region *C* in embryo of 12 days. To left of dotted line are normal muscle cells, to right are degenerating muscle cells, in the midst of which, and surrounded by dotted circle, are a group of muscle cells going through the first steps of transformation into electric tissue. × 1150.

Fig. 10. Transverse section of ventral electric spindle in process of formation in region *B*, of 12-day-old embryo of *Gymnarchus*. At left of figure two muscle cells begin to degenerate, while just to right of upper one of this pair a muscle cell is seen in advanced stage of atrophy. Connective-tissue capsule beginning to form. Electric muscle cells rather more coalesced than in fig. 9. × 1150.

Fig. 11. Transverse section of upper middle electric spindle in same section as fig. 10. Rapidly degenerating muscle cells to left. Further coalescence of electric muscle cells than in fig. 10. Myofibrils weakening but still in separate groups. × 1150.

Plate 4.

Fig. 12. Longitudinal vertical section of dorsal part of region *D* of 12-day-old embryo of *Gymnarchus*, showing relations of muscle tissue and electric tissue. Bracket 1 indicates zone of degenerating muscle fibers on inner median edge of five myotomes, on ventral side of median connective tissue; zone 3 shows atrophying muscle fibers on inner median edge of myotomes, dorsad of median connective tissue; zone 4, rudiment of upper median electric spindle; zone 5, same of dorsal electric spindle; zone 6, muscle tissue from ventral spindle to dorsal outer edge; zone 7, connective tissue; zone 8, dorsal epithelium. × 140.

FIG. 13. Longitudinal vertical section of ventral part of region *C* of 12-day-old embryo of *Gymnarchus*. Zone 1 indicates ventral epithelium; zone 2, ventral outer portions of five myotomes; zone 3, lower median electric spindle which is dividing into three parts in five vertebral segments; zone 4, inner degenerating muscle fibers of five myotomes; zone 5, projected outline of vertebral segments. × 140.

FIG. 14. Motor nerve cells from spinal cord of 42-day-old larva of *Gymnarchus*. *A*, *B*, and *C*, three electric motor nerve cells. *D* and *E*, two muscle motor nerve cells. From region *E*. × 1500.

PLATE 5.

FIG. 15. More highly magnified part of fig. 12. Brackets indicate same zones. × 760.

FIG. 16. Highly magnified part of transverse section through an electroplax from *C* region of 42-day-old larva of *Gymnarchus*. Curving bundles of modified myofibrils are seen in core and cut at various angles. Outer or electric layer is fixed and stained to show finer outer granules or electrochondria. Vacuoles as well as larger meshes of the linom are visible on inner edge of electric layer. Electric nerves are cut in sections at many angles and show medullary or connective-tissue sheath and its nuclei. At two points branching of these nerve fibers is visible and at four points its contact with electric layer of electroplax is to be seen. × 1500.

PLATE 6.

FIG. 17. Longitudinal section of a young electroplax from *B* region of 12-day-old embryo. Segmentation of electric tissue is now complete into unit electroplaxes of which figure is good example. Central fibril bundle beginning to lose its transverse striation. Structure is as long as three muscle segments or bony segments, as near-by spinal processes show. This represents oldest stage in embryo of this age. × 210.

FIG. 18. Longitudinal section of young electroplax from *C* region of 42-day-old larva of *Gymnarchus*. Fibrils have lost all striation and become waved in middle part. Cytoplasm shows strong differentiation at two poles (ends). Anterior end strongly vacuolized. Papillæ begin to grow from ends of middle part and nerve-endings have become established at junction of posterior third with middle third. This shows youngest stage in 42-day-old larva and is a successively later step in differentiation than fig. 17. × 210.

FIG. 19. Longitudinal section of an electroplax from *E* region of 42-day-old larva of *Gymnarchus*. Fibral bundle much "waved" in middle third, which now begins to show form of electroplax. Papillæ more developed. Nucleus and some cytoplasm inclosed in fibrillar core. × 210.

PLATE 7.

FIG. 20. Longitudinal section of another electroplax from further caudad in *E* region of same larva of *Gymnarchus*. This represents oldest stage accessible and is regarded as almost adult in its histological characteristics. Two nuclei shown in fibrillar core; one of these shows characteristic swelling.

PLATE 8.

FIG. 21. Transverse section of body of 42-day-old embryo of *Gymnarchus* from *D* region. Greater part of muscle tissue is here transformed into electric tissue. Section passes through very last part of dorsal fin. Electroplaxes cut at different levels and in one case section passes through right ventral spindle showing only electric connective tissue. *D*, dorsal spindles; *U.M*, upper middle spindles; *L.M*, lower middle spindles; *V*, ventral spindles; B_1 and B_2, caudal blood-vessels; *N*, neural canal. N_1, N_2, N_3, and N_4, electric nerves; N_5, lateral line nerve. × 110.

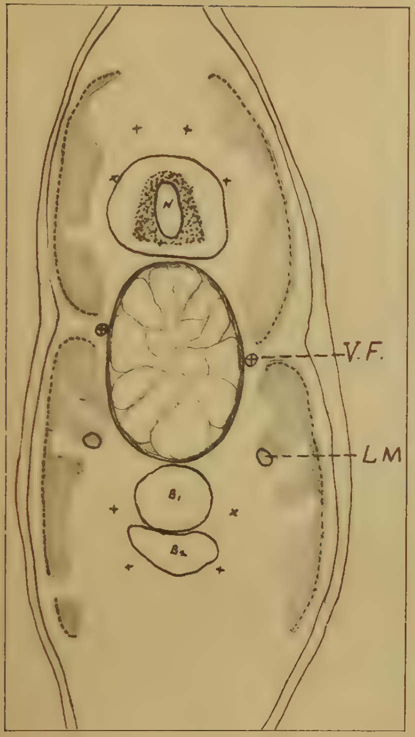
FIG. 22. Part of transverse section from middle part of an electroplax in *E* region of same larva of *Gymnarchus*. This figure serves well to show larger granules that lie deeper in electric layer, also connective-tissue sheath or wall of electric spindle. × 1500.

PLATE 9.

FIG. 23. Longitudinal section from *E* region, showing parts or whole of 11 electroplaxes lying in dorsal, upper middle, and lower middle spindles. Some remaining muscle fibers are visible at sides. Figure serves to show that there is no heavier, transverse connective-tissue wall lying in electric connective tissue, as is found in *Mormyrus*. This emphasizes secondary segmentation found in *Gymnarchus*. × 110.

FIG. 24. Small part from edge of basal part of a papilla on posterior surface of an electroplax from *C* region of 42-day-old *Gymnarchus.* Shows a single nerve, ending in a somewhat elongate and branched end-plate in electric layer. Outer crowded mass of granules stained deeply with eosin; larger inner granules not stained. × 1500.

FIG. 25. Small part of edge of another part of anterior surface of same electroplax. Shows a nerve fiber dividing and each branch ending in typical club-shaped nerve ending of *Gymnarchus.* × 2200.



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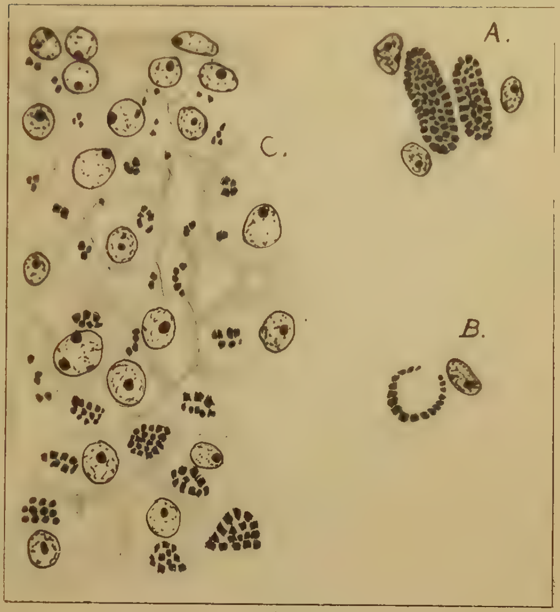

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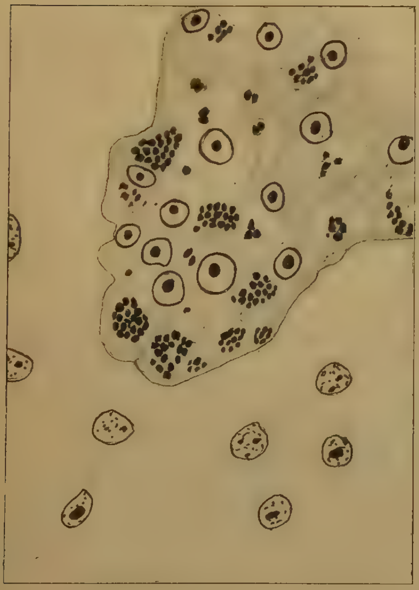

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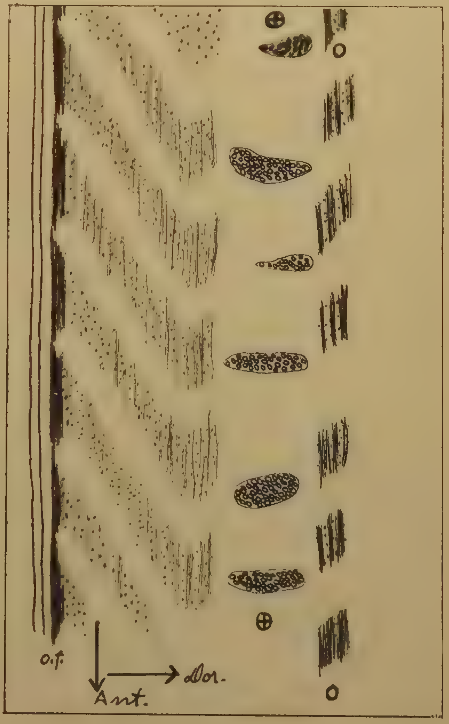

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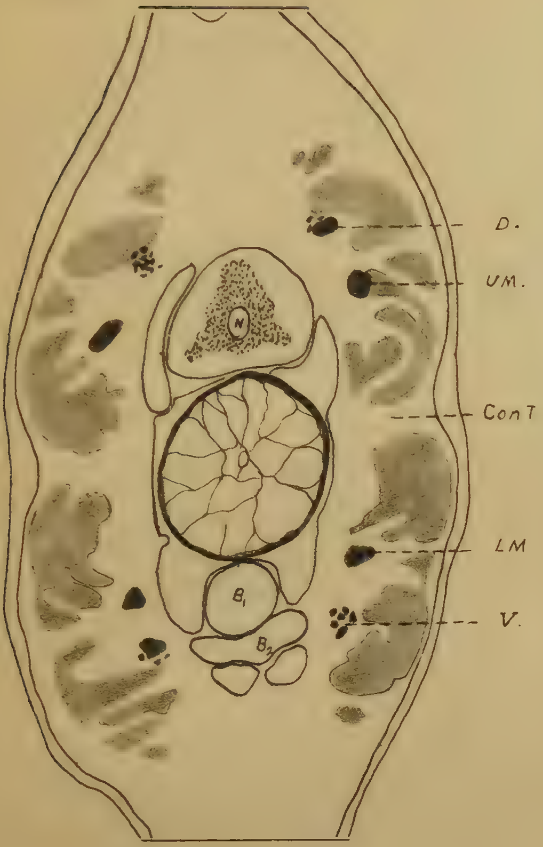

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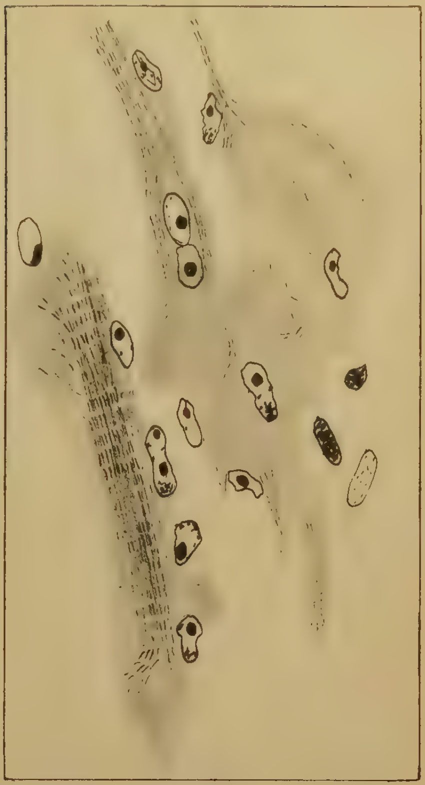

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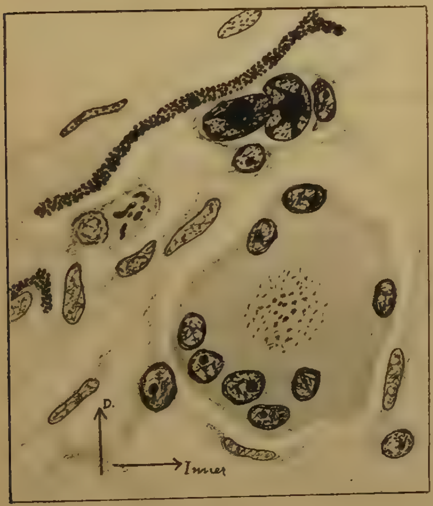

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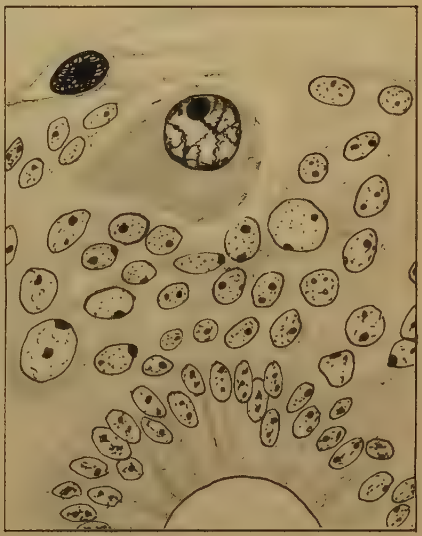

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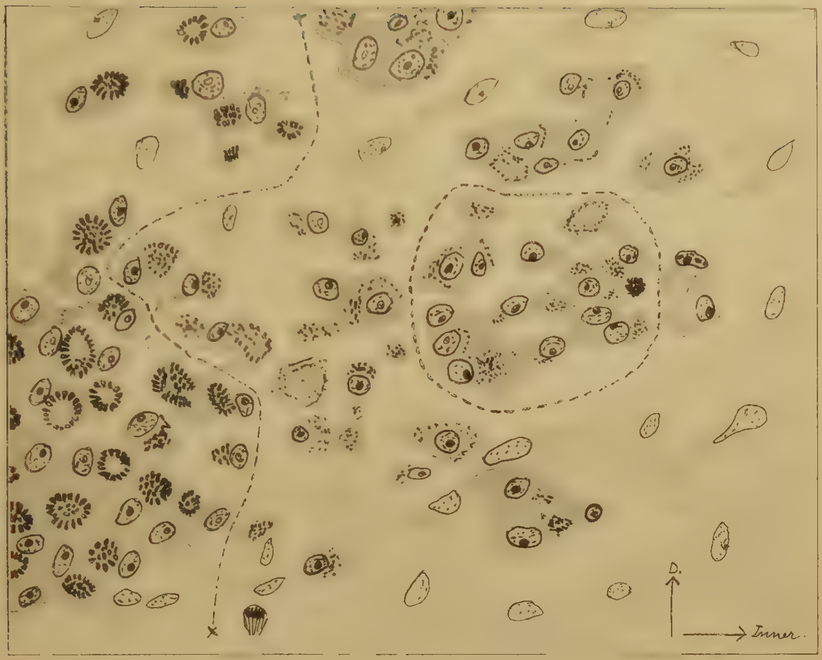

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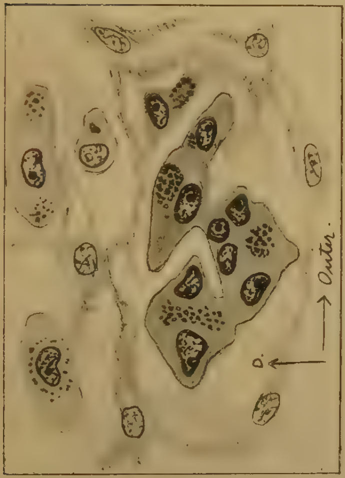

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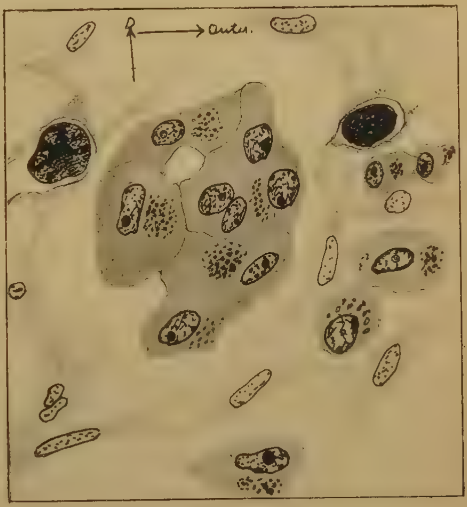

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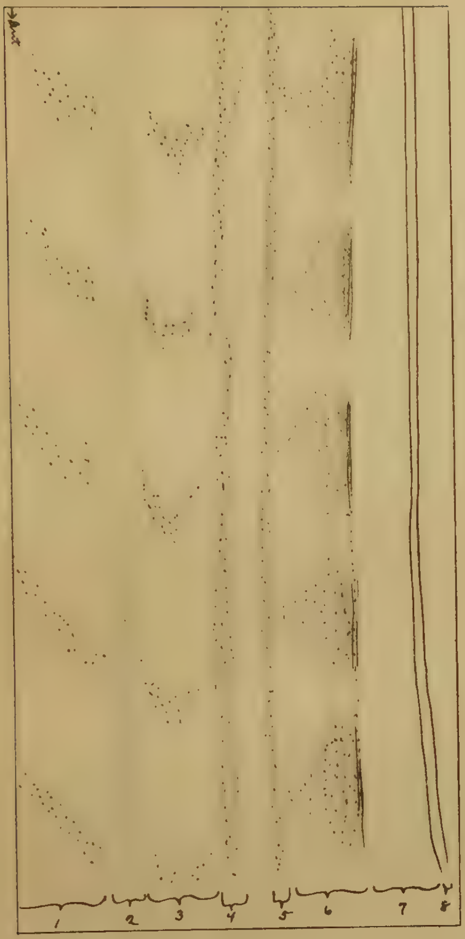

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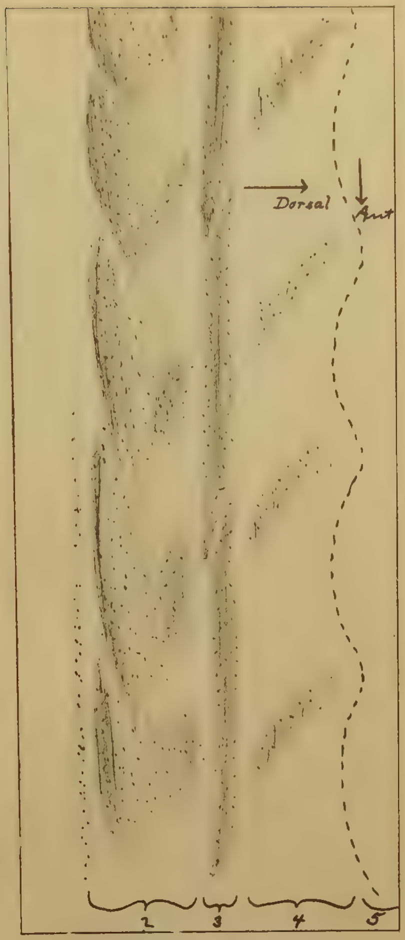

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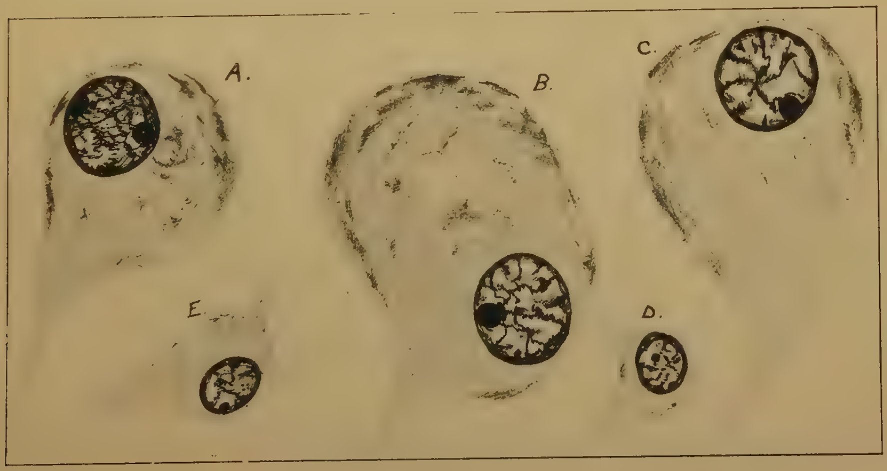

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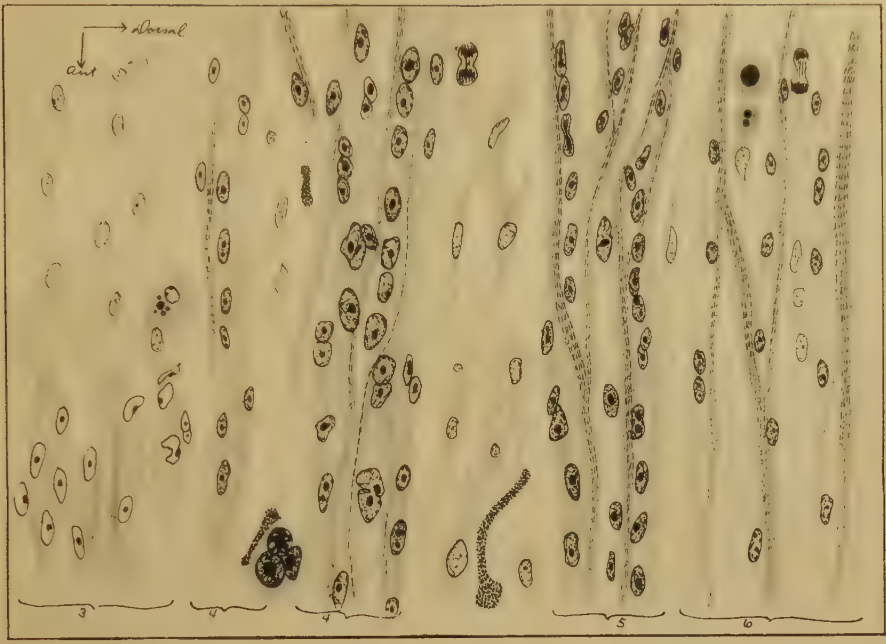

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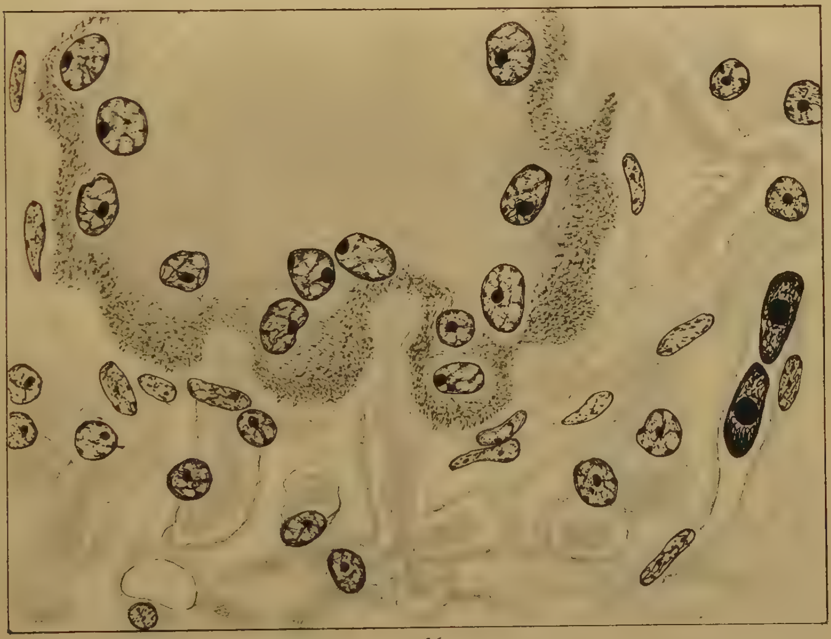

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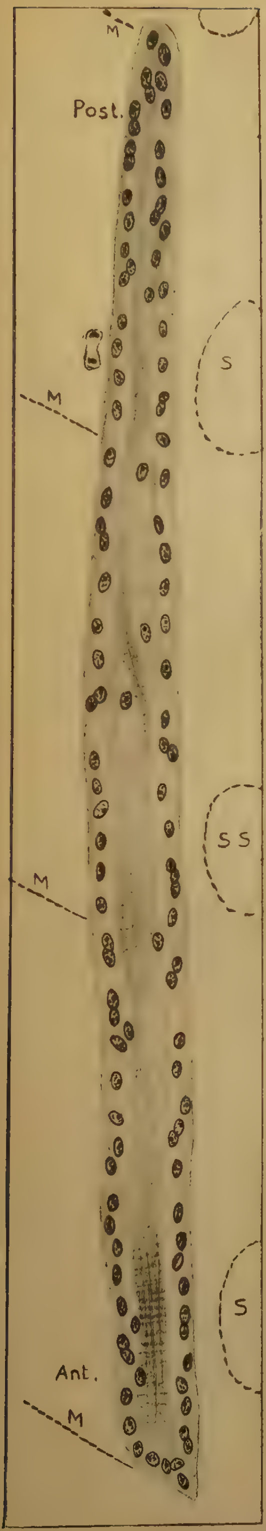

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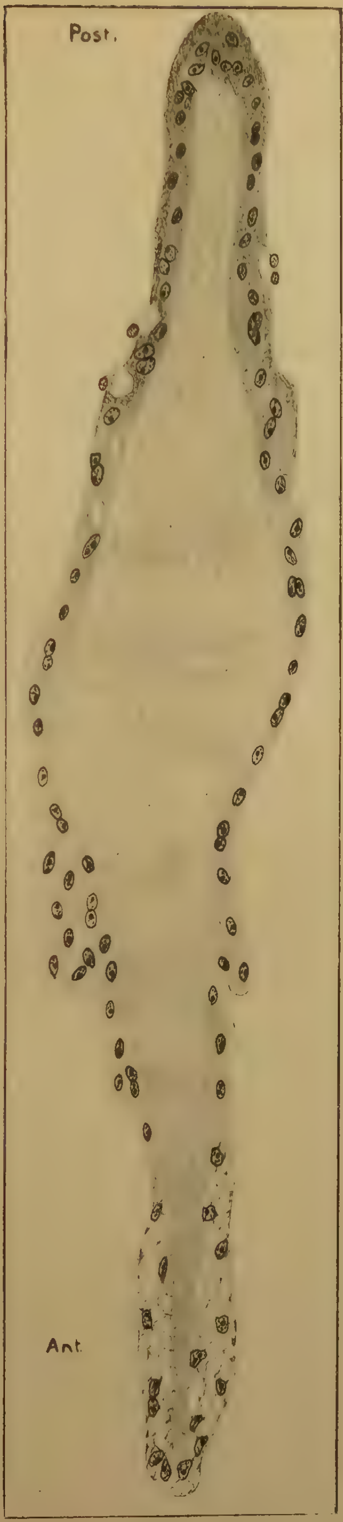

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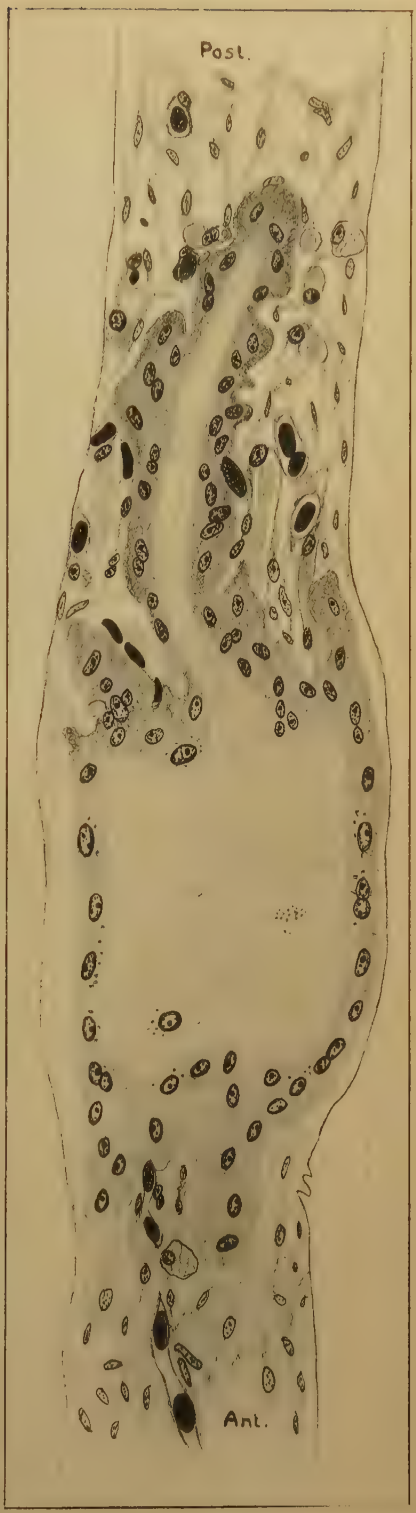

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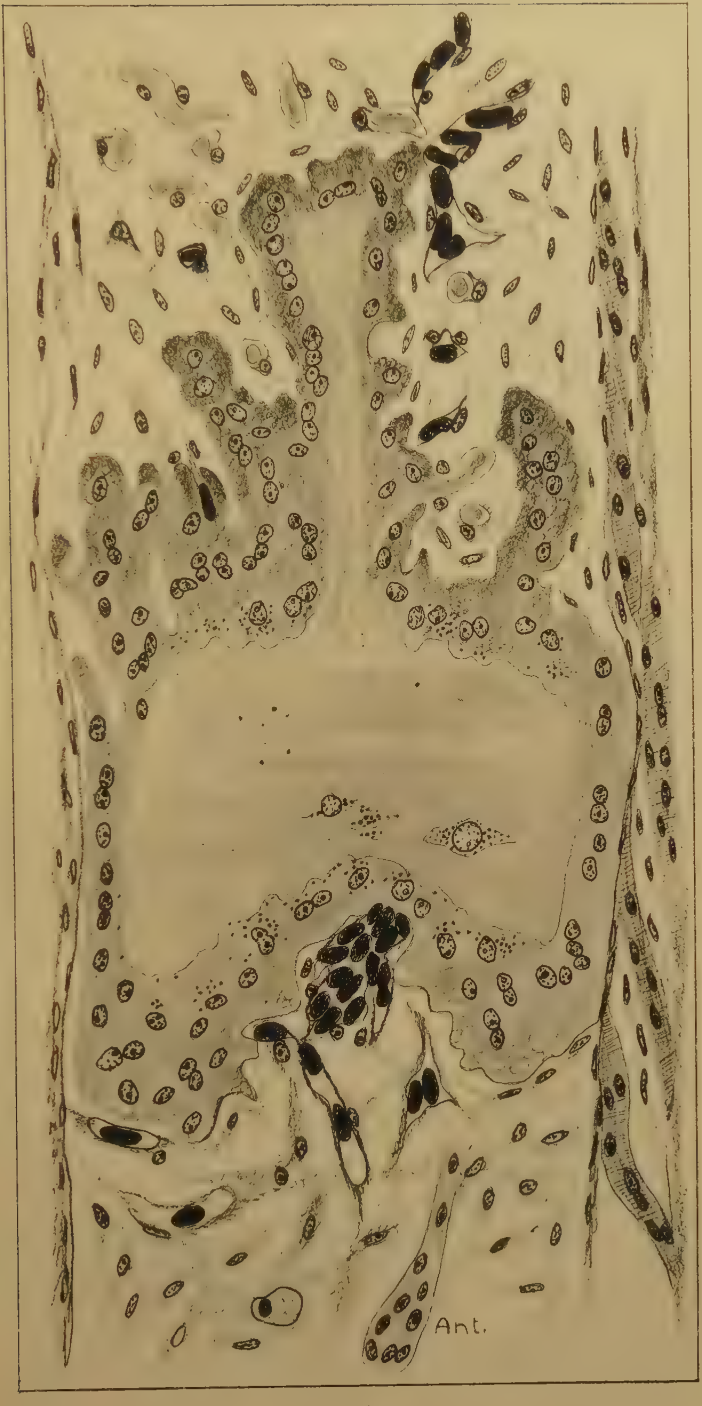

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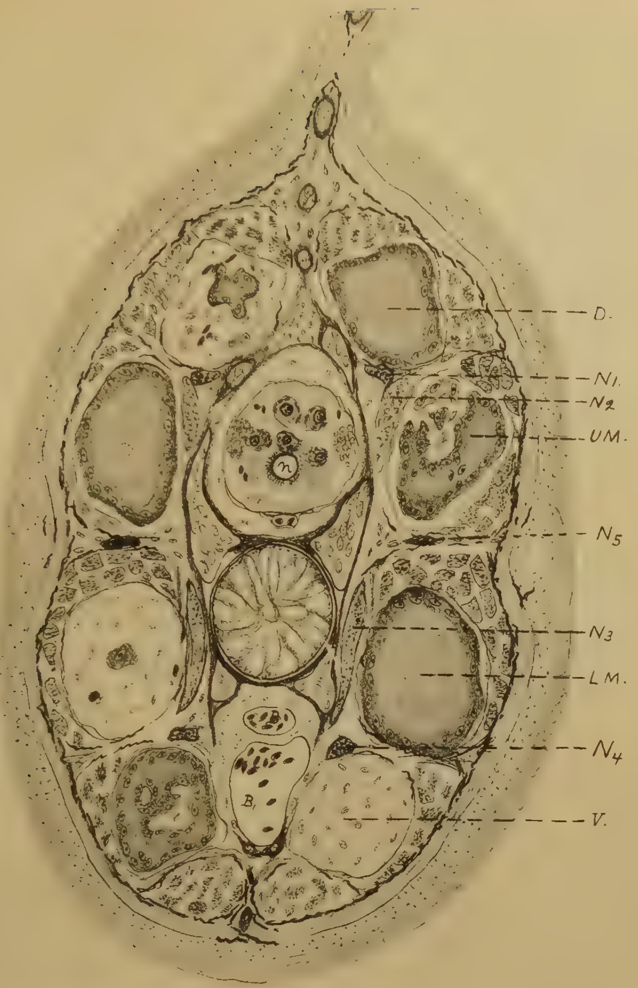

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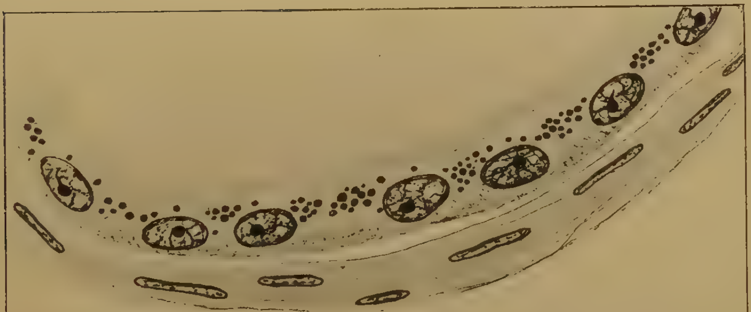

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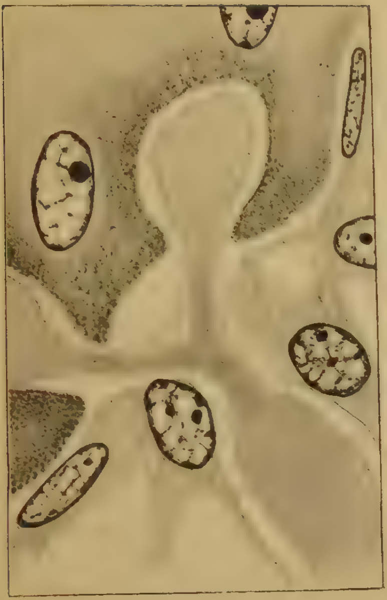

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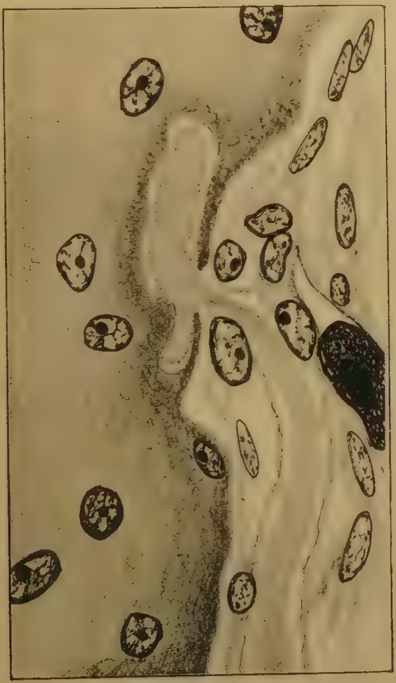

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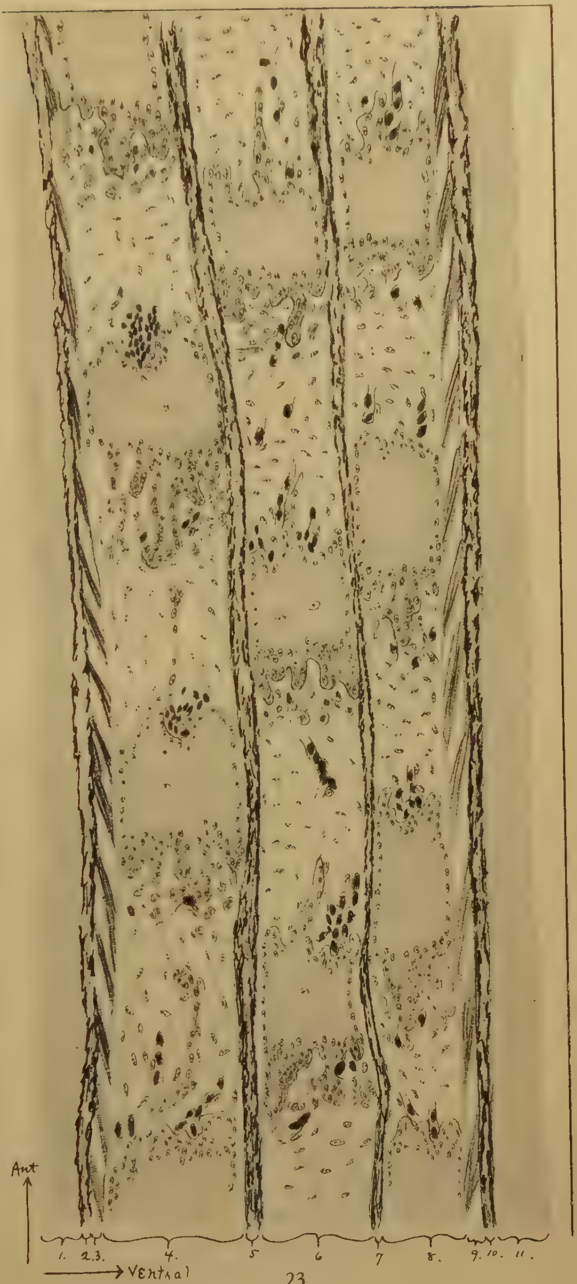

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XI.

THE DEVELOPMENT OF THE APYRENE SPERMATOZOA OF STROMBUS BITUBERCULATUS.

By EDWIN E. REINKE.

Proctor Fellow in Biology, Princeton University.

Seven plates.

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THE DEVELOPMENT OF THE APYRENE SPERMATOZOA OF STROMBUS BITUBERCULATUS.

By Edwin E. Reinke.

INTRODUCTION.

The fact that there is a dimorphism in the male sex cells of many Prosobrachs is well known at the present day. This is due to the great number of investigations that have been directed to the subject, but, in spite of them all, we are still as much in the dark as regards the function of the atypical spermatozoa as were the earlier investigators who did their work more than half a century ago. Furthermore, while the list of Prosobranchs in which this dimorphism obtains has been extended to a great many forms, in only a few of them do we have careful descriptions of the structure of the atypical spermatozoa, and in only one case (*Paludina*) is there a complete account of their development.

Obviously the solution of the problem is to be sought in a study of as many forms as possible, for I believe that each form has not only its own peculiarity in structure, be it great or small, but also its peculiarity in development. By peculiarity I mean not only the differences in form and size and in development between the typical and the atypical spermatozoa of any one genus, but also the differences between the atypical spermatozoa of different genera. In a comparative study of these peculiarities and differences may be found the explanation of the existence of the dimorphic spermatozoa. Comprehensive work such as this has already been begun; Kuschakewitsch ('11) has reported briefly upon the first two of a series of forms under investigation by him, and the writer has recently done the same for *Strombus*.

My attention was first called to the question of the dimorphic spermatozoa in Prosobranchs by Professor E. G. Conklin. At his suggestion *Urosalpinx cinerea* was made the main object of the investigation, supplemented by comparisons with the fresh-water snails *Goniobasis virginica* and *Paludina vivipara* (the Japanese variety). Material was collected and considerable work was done on these forms. But in May 1911, while at the temporary laboratory of the Carnegie Institution of Washington established at Port Royal, Jamaica, I was afforded the opportunity of studying and collecting material of various Prosobranchs, including *Strombus bituberculatus*. This form proved so interesting and so favorable for research that my immediate attention was centered upon it. My work was further advanced by another visit, in March 1912, to the temporary laboratory of the Carnegie Institution, this time at Montego Bay, Jamaica. Here I was able to carry on with partial success some experimental work and also to verify in the living cells most of the stages in the development of the apyrene spermatozoa that I had traced in preserved material.

My thanks are due to the Carnegie Institution for the privileges extended to me on both occasions, and in particular to Dr. A. G. Mayer, the Director of the Department of Marine Biology. Dr. H. A. Pilsbury of the Academy of Natural Sciences of Philadelphia very kindly identified the species for me. I am under great obligation to Dr. L. R. Cary, of the Department of Biology, Princeton University, for turning over to me his sectioned material of the testis and oviduct of *Goniobasis*. Finally, I wish to express a very sincere gratitude to Professor Conklin, not only for his many invaluable suggestions and criticisms, but also for the keen interest which he has at all times taken in the work.

HISTORICAL.

In 1837 v. Siebold discovered the fact that there are two kinds of spermatozoa in the testis of *Paludina vivipara*. The typical ones he called hair-shaped and the others worm-shaped spermatozoa. Since then the question of this dimorphism has been investigated by several writers nearly all of whom have given their attention mainly to *Paludina*.

Paasch ('43) and Kölliker ('41 and '47) denied the existence of the so-called worm-shaped spermatozoa of v. Siebold, the former interpreting them as being bundles of the hair-shaped spermatozoa and the latter claiming them to be different stages in the development of a single kind of spermatozoon. Leydig ('50), however, not only agreed with v. Siebold as to the existence of a dimorphism in the spermatozoa of *Paludina*, but also corroborated his description of the development of the worm-shaped kind. His views were very generally accepted until Duval ('80) investigated the question. He described an independent origin for the two kinds of spermatozoa, but in other respects his work was shown by v. Brunn ('84) to be quite inaccurate.

Although entirely confused in the successions of cell-generations, v. Brunn made a nearer approach to the facts in the case than anyone had done previously. He also described the dimorphic spermatozoa in five genera besides *Paludina* and later in the same year extended the entire list to include the following forms: *Paludina vivipara*, *Ampullaria* (sp.?), *Murex brandaris*, *M. erinaceus*, *M. trunculus*, *Cerithium vulgatum*, *Nassa mutabilis*, *Fusus syracusanus*, *Marsenia* (sp.?), *Aporrhais pes pelicani*, *Cassidaria echinophora*, *Dolium galea*, *Tritonium cutaceum*, *T. parthenopeum*, *Vermetus gigas*.

Brock ('87) added to this list by describing the atypical spermatozoa in three exotic Prosobranchs, *Pteroceras lambis*, *Strombus lentiginosus*, *Cypræa annulus*, *C. caput serpentis*, and *C. lurida*. As regards *Strombus*, his descriptions and observations are quite accurate as far as they go, but his figures are lacking in detail.

There then followed two papers, one by Köhler ('89) on *Murex*, and the other by Auerbach ('96) on *Paludina*. These authors established the fact that there is an almost complete loss of chromatin during the development of the worm-shaped spermatozoa, but they too were unable successfully to trace the succession of cell-generations and thus they made little or no material advance over the work of v. Brunn. To these observations Erlanger ('97) added a minute description of the adult worm-shaped spermatozoon in *Paludina*.

It is unnecessary here to go into a detailed discussion of the literature mentioned above, as it has all been discussed in the work of Meves ('03). This author has given a detailed and correct description of all the various stages in the development of both kinds of spermatozoa in *Paludina*. Furthermore, he has given careful consideration to the findings of the more important of the earlier writers and has shown wherein they were in error.

Meves describes the earliest differentiation between the two kinds of spermatozoa as occurring during the growth period of the spermatocytes, the spermatogonia of both being alike. He denies the statement of v. Brunn that the basal nuclei reproduce by direct division. This seems to the writer to be a debatable ground and forms the only weak point in the work of Meves. v. Brunn, of course, was quite wrong in supposing that the cells formed by this direct division of the basal nuclei give rise eventually to both kinds of spermatozoa; he failed to recognize the spermatogonia as distinct primitive elements of the testis. According to Meves these cells undergo unequal growth and thus give rise to both the hair-shaped and the worm-shaped spermatozoa. At the suggestion of Waldeyer he uses the terms eupyrene and oligopyrene to describe the two kinds of spermatozoa. The words have reference to their adult condition, *i. e.*, those provided with the ordinary amount of nuclear material and those with but a little; similarly, apyrene spermatozoa are those in which the entire nucleus disappears.

According to Meves, the oligopyrene spermatocytes of *Paludina* grow very much larger than the eupyrene and are clearly distinguishable, not only on account of their increased size, but also on account of their peculiar pattern and the presence of a large centriole and sphere. Preparatory to cell-division, this centriole divides and a little later both halves fragment into many smaller centrioles. The division which now occurs is abnormal. Two groups of very small centrioles form the poles of an ill-defined spindle. The daughter chromosomes pass to both poles, but before the division of the cell-body is completed the majority of them scatter again throughout the cytoplasm; four of the chromosomes, however, remain at the poles to form the new nuclei. These become vesiculated and may all unite with one another or some may unite and others remain single or all may remain single. Thus there may be four, three, two or only one nuclear vesicle in the secondary spermatocyte. The remaining ten chromosomes undergo a peculiar vesiculation in that the chromatin becomes lumped as a crescent on one side of the vesicle.

The second division follows immediately and is even more abnormal than the first. The centrioles scatter to the periphery and from each there pass out radiations. With the dissolution of the nuclear membrane or membranes, as the case may be, four new chromosomes make their appearance; these all show a longitudinal split. Very soon, radiations from a centriole are attached to each of the new chromosomes and they are drawn to various points near the periphery of the cell. By this time the ten vesiculated chromosomes remaining from the first division have been inclosed in a sort of capsule and are thus prevented from participating in the ensuing division. This is introduced by a slight elongation of the cell. Next, the centrioles move to the two ends of the cell and in so doing they form a spindle; one daughter chromosome then passes to each pole, while the other chromosomes remain scattered in the cytoplasm. With the division of the cell-body, the vesiculated chromosomes (inclosed in their capsule) pass into one or the other of the daughter cells, where they are set free by the dissolution of the capsule.

As a result of this division, Meves holds that the new nucleus is formed out of only a single chromosome, which very quickly becomes vesiculated and increases in size; all of the other chromosomes are gradually dissolved in the cytoplasm. Before the process of division is completed, cilia grow out from the centrioles, forming a brush at each pole of the dividing cell. After the cell has divided, the centrioles divide and their distal halves begin to move across the cell. A connection in the form of a fiber is always maintained between the proximal and the distal halves of the centrioles, the former remaining attached to the cell-wall. The nucleus in the meanwhile decreases in size and its chromatin forms a layer under the nuclear membrane. It moves closer to the plate of dividing centrioles. The continued movement of the distal centrioles across the cell results in pushing the nucleus against the cell-wall and in forming the bundle of axial fibers between the proximal and distal centrioles. Further growth of the axial fibers is accompanied by a gradual lengthening of the cell until the typical worm-shaped condition is reached. At the very end of the spermatozoon the nucleus forms a thimble-like cap over the end of the bundle of axial fibers.

In formulating an hypothesis to explain the problematical function of the oligopyrene spermatozoa, Meves discusses the theories of v. Brunn and Brock. v. Brunn believed them to be rudimentary eggs and therefore functionless, and this for two reasons: first, after careful investigation he failed to find them either fertilizing the ovum or even present in the oviduct; second, he believed their development bore resemblances to that of the ova. These views Brock attempted to refute on the grounds that any such rudimentary structure must have a proved homology with some still functioning part or organ and its departure in structure and development must be shown to be due to retrogressions; furthermore, if the dimorphism of spermatozoa is to point to an ancestral hermaphroditism, as v. Brunn claimed, the lower Prosobranchs should show this peculiarity, which they do not. He comes to the conclusion, then, that they are functionless.

Now Meves has shown that the oligopyrene spermatozoa of *Paludina* in their development show a marked parallelism—not to the ova, but to the true spermatozoa, and that they are produced in numbers equal to the latter. He suggests that there may be certain conditions under which they do fertilize the eggs; granting that they do and since the nucleus is accepted to be the bearer of heredity, then an embryo resulting from an egg fertilized by an oligopyrene or apyrene spermatozoon would show preponderating female characteristics. Such a case would prove the hypothesis that the nucleus is the bearer of heredity. The spermatozoon would then have, either primarily or solely, the mere function of stimulating the egg to division. According to Boveri, this stimulus is due to the introduction of a centrosome into the egg. But if it should be shown that actually the oligopyrene spermatozoa never fertilize the egg, then Meves would take the view, with Brock, that they are functionless.

Stephan ('03*a*) investigated the apyrene spermatozoa of *Cerithium vulgatum*, *Murex trunculus*, *M. brandaris*, *Triton nodifer*, and *Nassa mutabilis*. All of these forms he reported to have apyrene spermatozoa whose development is comparable to that of the oligopyrene of *Paludina*. All of the chromatin disappears in the atypical spermatozoa of these forms and in every case except *Cerithium* the bundle of axial fibers never comes into contact with an existing nucleus, as is the case in *Paludina*. In *Cerithium* the advancing bundle of axial fibers does touch a small nucleus which is the equivalent of a chromosome. Before the fibers have completed their growth, however, this small nucleus degenerates and disappears completely. Again, in *Cerithium*, as in *Paludina*, the cilia persist, while in the other forms they are retracted before the spermatozoon has fully matured. Upon these facts and also upon the positions of the anterior centrosomal structure in the adult atypical spermatozoa of the different forms under investigation, he concludes that gradations may be established between the different types of atypical spermatozoa, with *Cerithium* occupying an intermediary position between *Paludina* and the others.

In another paper Stephan ('03*b*) described briefly the development of the apyrene spermatozoa of *Murex brandaris*. The development here agrees in general with the description given by Meves for the oligopyrene spermatozoa of *Paludina*. It differs in the facts that there is an intense vacuolization of the cytoplasm and that there is no nuclear element in the adult spermatozoon.[1]

Basing his conclusions on certain observations of Popoff which were published later, R. Hertwig ('05) expressed his belief that the oligopyrene and apyrene spermatozoa serve to fertilize the egg, but such fertilization would not be a uniting of the male and female nuclear components, and would stand on the boundary between parthenogenesis and true fertilization.

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[1] A third paper by Stephan ('03*c*) on the development of the apyrene spermatozoa of *Cerithium vulgatum* and *Nassa mutabilis*, with two figures, was inaccessible to me.

This fertilization would result, according to Hertwig, in the production of males: "The sex-determining capacity which is indicated in the female by the formation of large eggs that are rich in yolk and small rudimentary ones, in this case would be transferred to the male sex."

Retzius ('06) described and figured the atypical spermatozoa of *Aporrhais pes pelicani*, *Turritella terebra*, *Murex trunculus*, *Fusus despectus*, and *Buccinum undatum*.

Popoff ('07) made a series of very careful and important observations upon *Paludina*. He examined the seminal receptacles of a great number of females at all times during the period of their sexual activity (May to November). He found that any one of the following conditions may obtain: (1) The receptacle may contain normal eupyrene and oligopyrene spermatozoa; (2) it may contain normal eupyrene and degenerating oligopyrene spermatozoa; (3) it may contain only eupyrene spermatozoa; (4) it may be completely empty. Of the 476 females examined during the whole period, in 176 cases the receptacle contained normal eupyrene and oligopyrene spermatozoa; the proportion of the cases in this condition to the total number examined at any one time showed a decided fall after the period of greatest sexual activity was reached (July). The condition which obtained in the next greatest number of cases (152) was the fourth, in which the receptacle was empty, and here the percentages of cases increased as the season advanced. In 89 cases the receptacle was found to contain only eupyrene spermatozoa, *i. e.*, the third condition; the percentage of these cases fell during August and then held fairly constant. Finally, only 39 individuals were found to be in the second condition (normal eupyrene and degenerating oligopyrene spermatozoa in the receptacle); here the percentage of cases gradually increased until August and then fell in September.

These facts indicated that the oligopyrene spermatozoa are shorter-lived than the eupyrene. In order to ascertain this definitely, Popoff segregated a number of females from the males immediately after copulation. From time to time the seminal receptacles of several of these females were examined and as a result he was able to establish the fact that after 10 to 12 days the oligopyrene spermatozoa begin to degenerate, while this does not occur among the eupyrene until after about 25 days.

In addition to this, Popoff found both kinds of spermatozoa in equal numbers in sections of the oviduct; only in the case of animals whose receptacles contained no oligopyrene spermatozoa did he fail to find these forms in the oviduct. This would indicate that the function of the oligopyrene spermatozoa is to participate in the fertilization of the egg. His results, however, in attempting to obtain eggs at the time of fertilization were practically negative. In three eggs, which constituted the only clear cases, Popoff found that the spermatozoa which had entered were eupyrene, so that nothing could be stated with certainty concerning the function of the oligopyrene spermatozoa. He thinks, however, if they may be said to have any function with regard to the egg (as is indicated by their presence in the oviduct), that this function can only be of a sex-determining character.

Lams ('09)[1] reported briefly that the development of the atypical spermatozoa in *Murex* confirms the description given by Meves for *Paludina.* In sectioned eggs he was able to find only eupyrene spermatozoa and therefore came to the conclusion that they alone serve to fertilize the egg.

The experiments of Kuschakewitsch ('10) have led to some very interesting results. By obtaining the ripe eggs of *Aporrhais pes pelicani* from the oviduct and mixing them with a mass of both kinds of spermatozoa which had been diluted with sea-water, he was able to observe the maturation and the first two cleavages of from 10 to 40 per cent of the eggs thus treated. In many instances he could see the formation of an entrance-cone and the entrance of the spermatozoon itself into the egg, but in no case was he able to observe the same processes for the atypical spermatozoa. But in sections of eggs which had been killed 20 minutes after mixing with spermatozoa, he found that the apyrene spermatozoa can and do enter the eggs, either alone or in addition to the eupyrene spermatozoa. Here the apyrene forms undergo a series of degenerative changes and are finally extruded. In later stages, during and after the first maturation spindle, their presence in the egg has never been detected.

Here it is seen, then, that the apyrene spermatozoa were extruded before any nuclear changes had taken place in the egg. Again, in some Prosobranchs the apyrene spermatozoa are very large and totally immotile, so that one can hardly imagine that they can in any way get into the egg. In view of these facts Kuschakewitsch has come to the conclusion that any sex-determining function in the apyrene spermatozoa is to be doubted.

Finally, Kuschakewitsch ('11) has briefly described the development of the eupyrene and the apyrene spermatozoa in *Conus mediterraneus* and of the apyrene spermatozoa in *Vermetus gigas.* In both cases the development of the apyrene spermatozoa shows many differences from that of the oligopyrene of *Paludina.* All three forms, however, have in common the greatly increased growth of the spermatocytes as the starting-point of the differentiation between the typical and the atypical spermatozoa.

Kuschakewitsch finds that in *Conus* no divisions occur in the development of the apyrene spermatozoa and that the spermatocyte is transformed into the spermatozoon by the disappearance of all the nuclear material and the subsequent growth of two fibers and two flagella from the centrioles. The nucleus may either gradually dissolve as a whole or it may fragment. The process of fragmentation may take place in either of two ways: the nucleus may become vesiculated and then break down into two and later into several compact and vacuolated spheres, or it may become compact and break down into a number of dense chromatic bodies which probably represent chromosomes. In either case the end result is the disappearance of all the chromatin. The two fibers eventually come to lie at the surface of the cell and the flagella with the pair of centrioles belonging to them at one of the ends of the spermatozoon. The conduct of the mitochondria is very striking; they appear first as granules, but eventually form fibers. During the course of development one or more vacuoles appear in the cell, which are quite characteristic of the adult spermatozoon.

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[1] I have been unable to obtain this publication and have therefore relied upon the review of it given by Kuschakewitsch.

In *Vermetus*, too, he finds that there is a direct breaking down of the nucleus of the spermatocyte. The chromatin forms into karyomerites and upon the dissolution of the nuclear membrane these lie free in the cytoplasm and are all gradually absorbed. In the spermatocyte there is to be seen a pair of centrioles lying in a thicker mass of cytoplasm; these form a group of secondary centrioles at one pole of the cell at the time of the dissolution of the nuclear membrane or immediately afterwards. The secondary centrioles give rise to a number of threads which eventually form a bundle of axial fibers whose middle portion passes through the center of the cell and whose ends extend beyond it in either direction. In the cytoplasm of the cell there arise a great number of thick-walled chambers whose cavities are filled with a clear substance. In each of these chambers a sphere of albumen is gradually formed. In the spheres themselves there appears either a large central vacuole or several smaller peripheral ones. Afterwards the contents of these vacuoles seem to become greatly condensed.

In his conclusions Kuschakewitsch points out the fact that the development of the atypical spermatozoa in *Conus* and *Vermetus* does not present the same parallel to the development of the typical spermatozoa as Meves established in the case of *Paludina*. He thinks, however, that we are justified in speaking of these structures as "spermatozoa" when the fact is borne in mind that their origin is the same as that of the oligopyrene spermatozoa of *Paludina* and *Murex* and that it is hardly conceivable that, in the well-defined group of Prosobranchs, structures with a similar origin would have a different morphological significance in different members of that group. Thus the mode of the development of the apyrene spermatozoa in *Conus* and *Vermetus* can in no wise be regarded as supporting the hypothesis of v. Brumm, that these structures are rudimentary ova. On the contrary, in *Conus* true eggs appear in the testis in addition to the apyrene spermatozoa.[1]

As regards *Strombus* the writer has shown (Reinke '12) that here, too, the development of the apyrene spermatozoa takes place without any maturation divisions, and furthermore that there are no processes, such as the formation of chromosomes and abortive spindles, which could be described as surviving from a condition in which those divisions did take place. In addition there is the fact (which will be shown in the succeeding pages) that the apyrene spermatozoa are not derived from the spermatogonia. But in spite of these facts, there are good reasons for still applying the term "apyrene (oligopyrene) spermatozoa" to these structures. In the first place, the name is well established and has been used commonly, and the words "apyrene" and "oligopyrene" are sufficiently descriptive to distinguish them from the true or "eupyrene" spermatozoa. Then again, they are spermatozoa in the sense that they are products of the testis and are found in the seminal fluid along with the true spermatozoa.

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[1] Since the above was written there has appeared another paper by Kuschakewitsch giving a detailed account of the development of the eupyrene and apyrene spermatozoa in *Conus* and *Vermetus*. In it he states that the apyrene spermatocyte of *Conus* sometimes undergoes a division after the nucleus has disappeared.

In retaining these names the writer does not mean to imply any homology between the apyrene and the eupyrene spermatozoa. Indeed, on account of the absolute lack of homology the terms "apyrene spermatocyte" and "apyrene spermatid" must be abandoned. A spermatocyte is a cell which is derived directly, by a mere process of growth, from a spermatogonium, and a spermatid is one of the four cells resulting from the two maturation divisions which such a spermatocyte has undergone and which is then transformed into a spermatozoon. The cells which give rise to the apyrene spermatozoa of *Strombus* go through two distinct periods of development and at no time can they be homologized with either the eupyrene spermatocytes or spermatids.

The first of these periods starts at the time when the cell is first recognizable in the testis.[1] This period continues throughout all the stages of the gradual but great growth of the cell and ends with the breaking down of the nucleus and the simultaneous scattering of the centrioles. The second period may be said to begin with the division of the centrioles; it is a period of marked cytoplasmic differentiations and it ends, of course, with the formation of the fully matured apyrene spermatozoon. For the cell during the first period it is proposed to use the name "apyrene spermatoblast" and during the second period to designate it as the "apyrene spermatosome." Both of these words, "spermatoblast" and "spermatosome," have been used hitherto in various connections; neither of them has the restricted meaning of "spermatogonium" or "spermatocyte" and, as used here, they imply no homologies with either of those structures.

METHODS.

Several fixatives were used for preserving the testis and the uterus of *Strombus*, namely, Bouin's, Flemming's, Helly's, and Zenker's fluids. Of these, Flemming's fluid (strong solution) gave by far the best results. Small pieces of the tissue were fixed for from 6 to 8 hours and were then washed for at least the same length of time, usually over night. As a result of such fixation the osmic and acetic acids did not penetrate very strongly into the inner portions of the tissue, and here the preservation of the mitochondria, the spheres, and the fibers was all that could be desired.

Sections were cut 5 micra thick and were stained with iron hæmatoxylin, followed by a weak solution of erythrosin in 70 per cent alcohol, which had been tinged to a purple color by the addition of a small quantity of a concentrated solution of gentian violet. The gentian violet was added to provide a nuclear stain for such structures as had not retained the iron hæmatoxylin; it gave a differential stain to the spheres and thus proved to be of considerable value. This stain has been used subsequently by other workers in this laboratory with fair success. Sections of the uterus and the seminal receptacle were stained in the same way.

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[1] It may be well to state at this point that the writer has succeeded in tracing the origin of these back to the so-called basal nuclei of Platner. He has found that the nurse-cells in the testis of *Littorina* have a similar origin, although the developmental changes are less complicated. It was deemed best, however, not to present the evidence of this origin at this time, but to wait until the history of the basal nuclei had been followed, in all its details, not only in these forms but also in other Prosobranchs, both those which have dimorphic spermatozoa and those which do not. The scope of the present paper, therefore, is confined to a description of the development of these cells from the time when their nuclei have been completely differentiated from the basal nuclei and have been established in the syncytium of the testis along with the true spermatogonia, each one surrounded by its own definite cytoplasmic body.

Smears from the testis, the vas deferens, and the seminal receptacle were prepared by making thin films of the contents of those organs diluted with sea-water. The films were allowed to dry over osmic vapor, were washed in fresh water, and then stained with Delafield's or iron hæmatoxylin.

Sea-water was found to be the best artificial medium for studying the living cells. The contents of the testis or sperm-duct were mixed with sea-water and then a drop of the mixture was placed on a slide and surrounded by a complete ring of glycerine-gelatine. In this way, a cover-glass placed upon the drop was not only supported, but it was also given enough rigidity to permit the use of an oil-immersion lense. If care were taken to see that the ring of glycerine-gelatine was unbroken, practically no evaporation of the mixture would take place. Under these conditions the various cells of the testis would remain alive for at least 2 hours. Sometimes methylene blue was used as an *intra vitam* stain, but this was found to be quite unnecessary and to cause an earlier breakdown of the cells than would otherwise occur.

OBSERVATIONS.

ADULT SPERMATOZOA.

The eupyrene spermatozoa of *Strombus bituberculatus* resemble in general those of other Prosobranchs. They are relatively short when compared with the spermatozoa of *Fasciolaria*, *Littorina*, or *Neritina*. The perforatorium is short and conical, the head thick and spindle-shaped (fig. 7). The head and the middle-piece, which is very slender and passes almost imperceptibly into the tail, constitute about half the total length of the spermatozoon.

In marked contrast to them stand the apyrene spermatozoa (fig. 6). The term "worm-shaped" can in no wise be applied to these, as they are totally different from the oligopyrene spermatozoa of *Paludina*. They were first described by Brock ('87) in the case of *S. lentiginosus* and again more recently and in some detail by the present writer ('12) in the case of *S. bituberculatus*. While Brock's brief description is correct as regards the general facts, his figure of the adult apyrene spermatozoon is not in accord with the facts as they have been observed by the present writer in *S. bituberculatus*, and there is no reason to suppose that any very great variation occurs between the apyrene spermatozoa of the two species. In their structure, the apyrene spermatozoa of *S. costatus* are identical with those of *S. bituberculatus*, the only difference being in their respective lengths. Apparently Brock did not fully understand the structure of the undulating membranes and he failed to observe the very evident centrosomal crescent at one end of the spermatozoon.

The adult apyrene spermatozoon of *Strombus* is composed of a central spindle-shaped body, which is long and narrow and slightly flattened dorsoventrally, on the sides of which are attached two broad undulating membranes (fig. 6). At the anterior end of the cell-body there is a crescent-shaped plate of centrosomal origin; starting from the horns of this crescent the undulating membranes round out quickly to their maximum width, while posteriorly they gradually become narrower and finally end in a short, sharply pointed tail-piece. In the membranes, here and there, can be seen indications of the fibers of which it is composed, but both these and the centrosomal plate are brought out more distinctly in fixed and stained specimens.

The interior of the cell is filled with a number of large secreted polygonal bodies composed of an albuminous substance or substances, possibly of a deutoplasmic nature like the yolk granules in the nurse-cells in the testis of *Littorina* (Reinke '12). When treated with iodine these bodies fail to give the reaction for starch or glycogen. When a mass of apyrene spermatozoa are separated from the eupyrene and are subjected to the xanthoproteic test, there is a very strong and clear reaction indicating the presence of albumen. In sections stained with iron hæmatoxylin the secreted bodies show the characteristic "Spiegelfärbung" of yolk granules. These bodies are more or less regular in shape and position but they decrease in size toward either end of the cell. In the living spermatozoon, at its posterior end, there can be seen a small aggregation of mitochondria. No traces of nuclear material can be found except in fixed specimens; here they appear as occasional darkly staining granules of degenerating chromatin lying between the albuminous bodies.

The orientation of the apyrene spermatozoon has been established upon morphological grounds. In *Paludina*, that end of the oligopyrene spermatozoon which contains the remains of the nucleus, *i. e.*, the head, has been designated as anterior and this, too, is the end toward which the axial fibers have grown. As there is no nuclear head in the apyrene spermatozoon of *Strombus*, the direction of growth of the axial fibers must determine the poles of the cell. The blunt, rounded end is the one toward which the fibers have grown and is therefore anterior, while the narrow pointed one is posterior. In movement it is the morphologically posterior end of the spermatozoon which is usually directed forward, although occasionally the reverse is seen to occur.

The movement of the spermatozoon is caused by contraction waves which pass alternately down the two membranes. At first these contraction waves are long and slow, propelling the spermatozoon with an even, steady motion. They usually pass down the membranes in a postero-anterior direction, but occasionally a reversal takes place; it is then that the spermatozoon is seen to move with its anterior end directed forward. The movement of the spermatozoon is comparatively slow and is not long continued, for it soon attaches itself, by means of its tail-piece, to the glass slide or other object on which it is being observed. As soon as this occurs, the contraction waves pass down both the membranes simultaneously and they then become much shorter and faster. With the spermatozoon attached in this way, the membranes may continue to be active for a time which varies with the medium used. In sea-water, contractions of the membranes were still seen after two hours, in isotonic NaCl after five hours, but in isotonic $MgCl_2$ they were very quickly inhibited. It very frequently happens, however, that the tail-piece breaks off and the spermatozoon swims away with a very much more rapid movement than it had at first.

When the tail-piece begins to break it can be seen to be composed of a number of fused flagella; sometimes, as the spermatozoon moves away a few of these flagella still adhere to it. This probably explains the statement of Brock to the effect that a tuft of flagella, which is invisibile at first, is to be seen after the spermatozoon has been swimming about for a while.

The first indication of breakdown in the spermatozoon occurs among the albuminous bodies, in that they gradually disappear. The process is undoubtedly one of liquefaction, for they leave in their stead a brownish semi-fluid substance in which, however, may still be seen the outlines of these bodies. When this has occurred one can observe for the first time the myoneme-like striations that are present on the cell-wall. Accompanying the disappearance of the albuminous bodies, there is a general flattening of the cell-body and the membranes. Undulations of the membranes continue a long time after this flattening has taken place and lead eventually to a partial separation of the membranes from the cell-wall. After this their disintegration is rapid.

The shape of the apyrene spermatozoa of *Strombus bituberculatus* is very constant and the variations in size that occur are very slight. From the centrosomal plate to the tip of the tail-piece the spermatozoa measure about 90 micra; the width of the membrane is usually about 5 micra and the greatest diameter of the cell-body averages about 8 micra. In *S. costatus* the apyrene spermatozoa are much longer, measuring 120 to 125 micra, but the other dimensions are not increased proportionately; according to Brock's statement they attain a length of 180 micra in *S. lentiginosus*. The apyrene spermatozoa in *S. bituberculatus* and in *S. costatus* are greatly outnumbered by the eupyrene. Brock has estimated the numerical relation between them to be about 1 to 500 for *S. lentiginosus*.

Because of the failure to obtain ripe eggs of *Strombus* at the time of the year when the material for this investigation was collected (March to the middle of May), it was impossible to determine experimentally whether or not the apyrene spermatozoa have any function with regard to the eggs. Ripe eggs of several sea-urchins were to be had in great abundance, however, and those of *Tripneustes* were mixed with some of the contents of the sperm-duct of *Strombus*. The presence of the eggs had no effect whatever upon either kind of spermatozoa except in a few instances where the egg-membrane had been ruptured by the pressure of the cover-glass. In such cases the eupyrene spermatozoa were strongly stimulated and swarmed around the eggs in great numbers, but the apyrene spermatozoa in the immediate vicinity of the eggs were not affected in the slightest.

At the same time of the year the seminal receptacle of the female was found to be quite swollen and full of a whitish mass. When some of the contents was diluted with sea-water and examined it was seen that in addition to a large number of oval granules only the eupyrene spermatozoa were present. These latter were normal and very quickly became active. Of a dozen females which were examined, not one showed a single structure in the seminal receptacle which could possibly be identified as the remains of an apyrene spermatozoon. The explanation of this phenomenon was found only when sections of the seminal receptacle had been made and studied.

The uterus and seminal receptacle constitute that portion of the vagina which lies just beyond the orifice of the oviduct, beneath the mantle. The vagina itself is simply a groove running beneath the mantle, at its base and on the right side, from the orifice of the oviduct proper to the foot; it corresponds to the genital groove of the male. Just below the orifice of the oviduct, the upper fold of the vagina is much broadened and thickened and is covered by the lower wall which is correspondingly broadened, forming a contractile sheath or mantle. This thickened portion of the vagina is the true seminal receptacle; it is a gland comprised of a large number of convoluted tubules lined with a secretory epithelium. The space between the seminal receptacle and its sheath may be spoken of as the uterus. Lying around the seminal receptacle in a semicircle is the main portion of the nidamental gland; one branch of the latter extends down along the vagina for a certain distance. The ducts of the nidamental gland open into the vagina, just below the seminal receptacle. Further on down, and at the point where the lower branch of the nidamental gland ends, is the opening into the vagina of a large diverticulum, the *bursa seminalis*. Beyond this point the vagina traverses the foot as an unmodified groove.

In the sections mentioned above, it was found that there was a dense detritus in the uterus composed of eupyrene and apyrene spermatozoa. Apyrene spermatozoa with relatively few eupyrene scattered amongst them formed the inner portion of this detritus. Segregating themselves from the apyrene spermatozoa, the eupyrene had moved for the most part to the periphery of the detritus. A clear homogeneous substance separated them from the apyrene spermatozoa. Other eupyrene spermatozoa had moved on into the tubules forming the seminal receptacle and had there arranged themselves with their heads against the outer surface of the epithelial cells. Here they were undoubtedly receiving nourishment, as was shown by the vacuoles which had taken the place of the distal granules of the secretory cells. In five series of sections no traces of apyrene spermatozoa were found in the tubules nor was either kind of spermatozoa found in the upper portion of the oviduct.

The changes that the apyrene spermatozoa had undergone were quite remarkable. In some cases the spaces between the albuminous bodies were packed with very small darkly staining granules; in others these granules had replaced the albuminous bodies in a portion of the spermatozoon; and in others the albuminous bodies had entirely disappeared and the whole body of the spermatozoon was filled with the small granules. In a few instances the body of the cell presented the appearance of an empty, shrunken shell lying between the two undulating membranes. Clearly the process going on in the apyrene spermatozoa was concerned with the breaking down and ultimate exhaustion of the substance stored in the cell.

It is probable, then, that in the examination of those dozen females only the tubules of the seminal receptacle had been tapped, and not the uterus, a contingency that is highly possible, depending upon the point of the incision and its depth. If that were so, then the granules observed were from the secretory granules of the tubules. But it is also possible that the uterus itself had been tapped and that the apyrene spermatozoa had undergone katabolic changes to such an extent that they were no longer recognizable.[1]

OBSERVATIONS UPON THE LIVING CELLS OF THE TESTIS.

By treating the contents of the testis in the manner already described, one can make a very comprehensive, if not detailed, study of the development of the apyrene spermatozoa of *Strombus*. This is particularly true of the later stages, where such structures occur as are represented by figures 1 to 5. These five figures, together with figure 6, show four very characteristic stages of the developing spermatosome from the time the axial fibers have grown across the cell up to and including the adult spermatozoon. They give a general idea of the appearance of the living cells and of the extent to which details of structure can be made out in them with the aid of an oil-immersion lens (Zeis 2 mm. apochromatic); furthermore, they bring out contours and the size relations existing between various stages to the greatest possible advantage.

In studying the living cells it was the hope of the writer not only to verify the stages which had been traced in fixed and sectioned material, but also, if at all possible, to watch the processes of growth and differentiation as they were taking place. All attempts, however, to observe the breaking down of the nucleus and the division of the centrioles proved to be unsuccessful. These processes are completed very rapidly and it is doubtful whether the centrioles with their radiations could be distinguished *intra vitam*. On the other hand, however, such processes as the vesiculation of the nuclear fragments, the formation of the albuminous bodies, and the growth of the axial fibers take place so gradually that they showed no appreciable progress in cells which were under constant observation for two or three hours. Nevertheless, many different stages in the development of the apyrene spermatozoa could be distinguished and observed most satisfactorily.

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[1] While a guest at the Laboratory of Marine Biology of the Carnegie Institution at Dry Tortugas during June 1913, the writer had the opportunity of examining some 25 females of *Strombus gigas*, all of which had copulated. While it is too late to present a detailed account of this examination, it may be stated that the results obtained give ample confirmation of the statements made above. The fates of the eupyrene and of the apyrene spermatozoa after copulation are very different. The eupyrene spermatozoa reach the seminal receptacle and are stored there in great numbers, remaining alive for an unknown period. Some of the apyrene spermatozoa move into the uterus along with the eupyrene, but there a sharp separation of the two kinds takes place, the eupyrene spermatozoa moving into the seminal receptacle while the apyrene clump together and undergo degenerative changes. Eventually the uterus becomes rid of them. But the greatest portion of the apyrene spermatozoa never even reach the uterus; instead, together with some eupyrene spermatozoa, they move into the *bursa seminalis* and there undergo degeneration until they are completely broken down. Gradually the whole mass is encapsulated in a secretion from the walls of the *bursa seminalis* and is finally extruded. In some instances it was found that the contents of the extruded capsule had become infected and that putrefaction had set in. There are some grounds for the writer's belief that some of the eupyrene spermatozoa which get into the *bursa seminalis* find their way out again and, passing up the vagina, reach the seminal receptacle. The most significant point is that oviposition had not yet begun.

The description given above of the relations of the various parts of the female genitalia is based upon dissections of *Strombus gigas*.

The large nucleated cell, corresponding to the oligopyrene spermatocyte I of *Paludina*, was easily recognized. Earlier stages in the growth of that cell could not be distinguished from the eupyrene spermatocytes. The next stage (fig. 1) which could be easily picked out was one in which the axial fibers had grown well across the cell and many of the nuclear fragments had become vesiculated. Figure 2 shows a stage of approximately the same age, but viewed at an angle of 90° from the other. In these stages the cell is not much larger than it was just previous to the breakdown of the nucleus. Later on, the continued growth of the axial fibers results in the elongation of the cell; this makes it appear larger, but no real increase in its volume has taken place. This is shown in figures 3 and 4, which represent stages following closely one upon the other and viewed in planes at right angles to each other. In figure 3 the bundle of axial fibers is seen just in the act of splitting apart, while in figure 4 the process is represented as being probably a little more advanced.

This splitting of the bundle of axial fibers is followed by the further separation of the halves until they reach the cell-wall on both sides. Very soon they begin to project edgewise beyond the cell, giving the first indication of the formation of the undulating membranes (fig. 5). Beyond this point there is not a very great increase, if any, in the actual volume of the cell; it merely becomes gradually longer and narrower until the adult condition is reached (fig. 6). It will be noticed that as the cell approaches maturity it becomes more and more filled with the albuminous bodies, while the nuclear fragments degenerate and disappear. When first formed, the albuminous bodies are spherical in size and are scattered irregularly throughout the cell, first filling up its anterior portion. Later on, as a result of their increase in number and the lateral compression of the cell due to its elongation, they become polygonal and arrange themselves in an orderly fashion. Until the undulating membranes have been formed the cell is immotile; the flagella at its base never beat, but instead undergo fusion to a certain extent. As will be shown later on, the myoneme-like striations are not present until after the formation of the membranes.

The study of the living cells of the testis is very valuable, because by this method all possibilities of abnormality due to fixation and subsequent treatment of the tissue may be eliminated. However, it leaves great gaps in the stages of development of the apyrene spermatozoa and affords very little or no chance of determining the finer details of the protoplasmic differentiations and the structural changes that occur in the cell. For this purpose the study of material that has been well fixed and stained is alone available.

GROWTH OF THE APYRENE SPERMATOBLAST.

In *Strombus* the large nucleated cell which becomes differentiated into the apyrene spermatosome can be traced back through a period of uninterrupted growth to a small cell, the apyrene spermatoblast, which is distinct from either the eupyrene spermatocytes or spermatogonia. This cell (fig. 8) lies close to the walls of the lobules of the testis and may be surrounded by older cells of the same nature or by the general syncytium of the testis or partially by both. Two or three of these cells, lying in the syncytium and isolated from others of their kind, are frequently in close proximity to a nest of eupyrene spermatogonia and would be taken at first glance to belong to that nest. Closer and more careful examination, however, reveals certain distinct, if minute, differences between them and the spermatogonia.

In the first place, there is a difference of position. Where they occur, the young apyrene spermatoblasts always lie between the wall of the lobule and the spermatogonia. If the group of apyrene spermatoblasts is a larger one, it is seen that the outer cells are larger and older than those lying next to the wall of the lobule. Such a group as that may be surrounded by several nests of spermatogonia or by spermatocytes. A comparison of all the nuclei of all these various cells shows that those of the outer spermatoblasts are as large or larger than the spermatogonial nuclei, while those of the inner spermatoblasts are smaller. But the cytoplasmic bodies of even the youngest spermatoblasts are larger than those of the spermatogonia and they are inclosed by definite membranes. The cytoplasm of the spermatogonia forms a thin sheath around their nuclei and the cell-walls are so delicate that very often it is impossible to distinguish them in the cytoplasm of the surrounding syncytium.

The nuclei of the apyrene spermatoblasts and of the eupyrene spermatogonia differ in pattern. In the latter, during the resting stages, the chromatin forms an irregular and dense network, the strands of which show many free ends and, when seen in cross-section, have very frequently the appearance of granules. Once the spermatogonia have begun to grow, the nuclear pattern changes gradually until the typical spireme of the spermatocyte is formed. The nuclear pattern of the spermatoblasts, on the other hand, remains constant until a late period in the growth of these cells, when they can be easily distinguished from the eupyrene cells by their increased size. These nuclei are relatively rich in nuclear sap and poor in chromatin, the latter being present in the form of irregular granules which are partially connected by a chromatic material (figs. 8–14). At least one karyosome is always present in the nucleus.

Finally there are two cytoplasmic structures which aid in distinguishing the young apyrene spermatoblasts. The first of these is a very evident, darkly staining granule, whose derivation is unknown. Its position varies; it may lie just beside the nucleus or nearer the periphery, but it is always found in the proximal half of the cell. It usually appears to be surrounded by a clear area (figs. 8 to 12). Occasionally a cell is found which has two or three of these granules in it. The other structure is the centrosome. This appears at first as a distinct centriole with a few faint rays and it lies between the nucleus and the base of the cell (fig. 8). In other spermatoblasts, which are still so young that they might be confused with the spermatogonia, it may be seen that the centriole has divided into two and that these are surrounded by a sphere (fig. 9). That centrosomes do exist in the spermatogonia is proved by their appearance in mitoses, but the writer has never been able to identify them positively in the resting cells. The distinct appearance of the centrosomes (centrioles) in the young spermatoblasts is not at all surprising when it is borne in mind that their later history is quite complicated and that they play a very important part in the development of the apyrene spermatozoa. The other bodies are never found in the cytoplasm of the spermatogonia. The eupyrene spermatogonia greatly outnumber the apyrene spermatoblasts and, with the basal nuclei, they occupy the space next to the wall of the testis except here and there where they have been displaced by groups of the spermatoblasts. These groups occur at irregular intervals and the cells composing them vary not only in number but also in age.

The development of the apyrene spermatozoa from these spermatoblasts takes place, as has already been indicated, without a single division of the cell. The first period of this development covers a gradual growth of the spermatoblast, resulting in a tremendous increase in the size of the cell. This statement applies not only to the nucleus and cytoplasm, but also to the centrosome; the latter comes to be the most striking and interesting element in the cell.

The structure of the primitive spermatoblasts has been described. They are irregular in outline and are not pear-shaped or stalked as are the oligopyrene spermatocytes of *Paludina* (fig. 8). Usually, as they increase in size and are crowded away from the wall of the lobule by the formation and growth of younger spermatoblasts, they are set free and tend to become round (fig. 12). Occasionally a cell which has almost attained its full growth is seen to have retained a connection with the syncytium by means of a stalk, even after it has been crowded away (fig. 13); such a cell presents a close resemblance to the oligopyrene spermatocytes of *Paludina*. In some few instances two or three fully grown spermatoblasts may be seen lying side by side close to the wall of the lobule and only separated from it by a thin layer of syncytium. These cells were the last ones to be formed at that point and have undergone their entire growth without suffering displacement. Both in the cases of these and of the stalked cells there is no relation between them and the syncytium other than one of close contact. The syncytium has become exhausted and has come to form a narrow fibrillar layer between them and the wall of the testis. This shrinkage of the syncytium beneath the spermatoblasts is noticeable in much earlier stages (figs. 9, 10, and 11).

The growth of the nucleus keeps pace with that of the cytoplasmic body. In the very young spermatoblasts the chromatin has the appearance of irregular granules lying in a matrix of diffuse achromatic material. There is always a karyosome present (fig. 8). Practically the same nuclear structures are shown in figures 9 and 10 except that in the former the karyosome was just above the plane of focus and in the latter the greater portion of the nucleus was in the next section. The karyosome is very persistent and constant in its appearance; in sections which have been destained until the other structures of the nucleus have lost their stain, the karyosome presents the appearance of a thick ring inclosing a lighter colored substance. As the nucleus grows larger, there is an increase in the amount of achromatin present and in the number of chromatin granules (compare figs. 10, 11, and 12).

The increase in the number of these granules is due, probably, to their division. The subsequent growth of the resulting halves keeps them all of a fairly constant size. When the nucleus has reached its maximum size the chromatin granules begin to grow at the expense of the achromatin (fig. 14). Their growth continues until they are as large or larger than the karyosome, so that the latter becomes indistinguishable. Eventually, practically all of the achromatin is absorbed and the chromatin granules are retracted to the periphery of the nucleus, just beneath the membrane. Here they undergo fusion to a certain extent and form large lumps of chromatin, or karyomerites, of various sizes and shapes (figs. 16, 17, and 18). The interior of the nucleus is filled with a colorless nuclear sap (fig. 17). It is now ready to break down, but before describing this process it will be well to consider the growth of the centrosome.

The first indication of a centrosomal structure in the spermatoblast appears at a very early stage, in the form of a distinct, darkly staining centriole, from which two or three rays (fig. 8) pass out. The position of this centriole, near the nucleus and in the proximal half of the cell, is invariable. The formation of a sphere around the centriole is not noticeable until the latter has divided (fig. 9). In such a stage as this the sphere is quite distinct and rays from both centrioles can be seen. It is impossible to say whether the next stage (fig. 10) is reached by a division of the two centrioles into four or by their fragmentation into a greater number. The centrioles are very much smaller than they were and can not be counted with any degree of certainty. The sphere has grown no larger and the rays from the centrioles do not extend beyond its periphery. The centrioles now lie in a homogeneous substance which fills the center of the sphere and is more deeply staining than the latter. This substance represents the first formation of the centrosome proper.

Further multiplication of the centrioles is accompanied by their separation from each other and by the spreading of the substance of the centrosome (figs. 11 and 12). It becomes very difficult to distinguish the individual centrioles from the substance in which they lie, but several of them can usually be seen lying at various points on the edge of the centrosome. The outer sphere, too, has increased in size and about this time begins to resolve itself into an outer dark court and an inner clear court (fig. 12). A little later on, the centrosome, which is now considerably larger, becomes spherical and acquires a definite membrane and the rays become stronger and stronger (fig. 13). It reaches its full growth at approximately the same time as does the nucleus (fig. 14). From it there passes out in all directions a great number of strong rays, which extend to all parts of the cell; at the base of each lies a small centriole. Lying around the centrosome on all sides, but more particularly on the sides away from the nucleus, is a mass of mitochondrial granules (figs. 14 and 15). They are irregular in shape and size and stain deeply with iron hæmatoxylin. Figure 14 does not show all the mitochondria that were seen in the cell.

The formation of the mitochondria is rather sudden. They appear in the sphere shortly after the formation of the inner court and are found at first on all sides of the nucleus. Figure 13 represents approximately the stage at which they first appear; it was drawn from a cell which showed the effects of osmic acid to some extent and therefore does not represent either the mitochondria or the rays as fully as they might be seen in other cells. As the mitochondria increase in number and size, they gradually obliterate the outer court of the sphere, while the inner court grows wider and wider. The last traces of the outer court are shown in figure 15. With the appearance of the mitochondria, one loses sight of that body which was mentioned as being found in the cytoplasm of the young spermatoblasts (figs. 8 to 12). This body gradually grows larger and very pronounced, but later on it is impossible to distinguish between it and any of the larger mitochondrial granules.

Judging from the great number of cells which are found with a fully formed centrosome, the last stage in the growth of the spermatoblast must extend over a very long period. Gradually the mitochondria withdraw from the space between the centrosome and the nucleus and that half of the centrosome which lies toward the nucleus becomes more lightly staining and seems to lose its definite outline. On the other hand, the opposite half of the centrosome takes a deeper stain and it can actually be seen that the centrioles are massing themselves upon that side (fig. 14). At this time, one or two comparatively large granules are to be found in the centrosome; they are very constant in their appearance but no suggestion can be made as to their significance. Finally, those rays which traverse the space between the centrosome and the nucleus attach themselves to the nuclear wall (fig. 14); then follow, in quick succession, the retraction of the chromatin to the wall of the nucleus, the dissolution of the latter on the side toward the centrosome, the scattering of the karyomerites, and the disappearance of the centrosome with its rays.

The actual beginning of dissolution of the nuclear wall is shown in figures 15, 16, 17, and 18; these go in pairs, figure 15 with figure 16 and figure 17 with figure 18, representing consecutive sections through two different cells. In figure 15 the mitochondria lie for the most part above the centrosome, while the latter in turn is at a slightly higher focus than the karyomerites. These last, represented in the figure as large black bodies, lie free in the cytoplasm, into which also some of the nuclear sap has escaped. Many of the mitochondria have grown into short stout rods (chondrioconts). It will be noticed that the rays from the centrosome are becoming less evident. In figure 16 is shown the remainder of the nucleus; some of the nuclear sap and a few of the karyomerites are moving out into the cytoplasm. The further and complete scattering of the karyomerites is usually accompanied by the total disappearance of the centrosomal rays. But in figures 17 and 18 a cell is shown in which absolutely no traces of the centrosome or the rays could be found and as yet none of the nuclear material had escaped into the cytoplasm.

These figures were drawn very carefully and show all the structures visible in the cell. The mitochondria were rendered somewhat indistinct by the action of the osmic and acetic acids, but the same can hardly be said of any surviving centrosomal structure; for a younger spermatoblast lying beside this cell showed a distinct centrosome. The nuclear membrane has become very much thinner and at one place, on the side towards the mitochondrial apparatus, it has been dissolved (fig. 18). It would therefore seem that the same process, undoubtedly a chemical reaction, which brings about the disappearance of the centrosome also leads to the dissolution of the nuclear membrane. Of the two phenomena, the latter is completed the more slowly. The karyomerites are carried to various parts of the cell with the diffusing nuclear sap.

The greater part of the chromatin remains in what was originally the distal half of the cell, although some of the fragments move into the region occupied by the mitochondria. This distribution of the chromatin is illustrated in figures 19 and 20, which represent two consecutive sections through the same cell. That part of the cell shown in figure 19 constitutes the proximal half of the cell, while the distal half is shown in figure 20. The larger lumps of chromatin now break down into several smaller parts (figs. 20 and 22), and as a result of this fragmentation there is a more equal distribution of the chromatin throughout the whole cell (figs. 24 and 25, consecutive sections through the same cell). The ultimate karyomerites show an extreme variation in size, some of them being extremely small (fig. 22).

Kuschakewitsch ('11) has described a somewhat similar formation of karyomerites in the apyrene cells of *Vermetus*. Here the karyomerites apparently are all fully formed before the dissolving of the nuclear membrane. They are of a fairly uniform size and shape and many of them show a longitudinal split, thus leading to the belief that they are true chromosomes. Kuschakewitsch states that "the chromatin of the nucleus gathers into larger elements, which almost entirely fill up the interior of the nucleus (Kernraum). In some cases there are formed, transitorily, true chromosomes which exhibit a clear longitudinal split. Then the nuclear membrane disappears and the chromatin elements (karyomerites) lie free in the plasma." The outstanding difference between the processes relative to the breaking down of the nucleus in the two forms is the fact that in *Strombus* the ultimate karyomerites are not formed within the nucleus while the latter is yet intact. Furthermore, when finally established, they have an extreme variation in size, little uniformity in shape, and never show any indications of a longitudinal split. In short, unlike those of *Vermetus*, the karyomerites of *Strombus* can not be said to be chromosomes, nor is it at all certain that they represent an attempt on the part of the chromatin to form chromosomes.

The further history of the karyomerites is one concerned with processes leading to their vesiculation and, later, to their gradual degeneration and ultimate disappearance. When first scattered into the cytoplasm the karyomerites lie in close contact with it on all sides. Very shortly afterwards, a clear space, one which gradually grows larger, separates them from the surrounding cytoplasm (figs. 20 to 25). The substance filling this space is essentially like nuclear sap and is probably formed by a differentiation of the cytoplasm immediately surrounding a karyomerite. A little later a definite membrane can be seen to have been formed around the clear area, separating it and the inclosed karyomerite from the cytoplasm (figs. 26 to 32). It is quite possible that this membrane is formed as a precipitation membrane between colloids of opposite electrical charges. If this explanation is the true one, then the differentiation of the cytoplasm must have been caused by a chemical reaction between itself and the karyomerite, the substance formed acquiring the acid properties of the chromatin. The reaction must continue until the interposition of the formed substance causes too great a separation of the reacting substances. Then, when the reaction has ceased, a precipitation membrane is formed between the acid and therefore positively charged substance resulting from the reaction and the basic and negatively charged cytoplasm.

After the formation of the membrane, the inclosed karyomerite breaks down into several smaller pieces, each of which takes up a position just beneath the membrane (figs. 26 to 32). In this way a number of chromatic vesicles arise which are very characteristic for the ensuing stages, and which, as will be shown later, persist for a long time before their actual degeneration sets in. Most of the smaller chromatic fragments, however, do not form vesicles but are absorbed without any further changes.

The disappearance of the centrosome takes place very suddenly and the nature of the process is not entirely understood. Absolutely no structures were found which could be interpreted with any degree of assurance as being phases of the centrosome subsequent to the stage shown in figure 15. One cell alone showed an indistinct structure which might be taken for the remains of a rapidly dissolving centrosome, but this can not be asserted to be the case (fig. 19). The body was round and apparently denser at its periphery than in the middle and from it there passed off a number of short and rather vague rays; it was partially hidden by what is taken to be a large mitochondrial body. The figure of it is not presented because much importance is attached to it, but merely because it happened to occur in a cell which was valuable for other features which it showed. All that is definitely known to occur is that while the substance of the centrosome is dissolved and disappears very rapidly, the centrioles themselves are unaffected by the action. Having been freed, they scatter in small groups.

The movements of the centrioles are hidden to a great extent, as it is very difficult to establish their identity among the many and very small mitochondrial and chromatic granules present. They eventually reach the cell-membrane, however, either singly or in small groups, and here some of them can be discerned quite readily (figs. 19, 24, and 25); others of perhaps questionable identity can sometimes be seen in the interior of the cell (fig. 19). A little later, secondary rays arise from those centrioles which have reached the membrane, causing them now to become very evident (figs. 21, 22, 26, and 27). Figures 21, 22, and 23 are consecutive sections through the same cell, and that portion of the cell represented by figure 21 showed the majority of the centrioles; those lay beneath the plane of the focus figured. A few others were found in the next section of the cell (fig. 22), but none were found in the upper section (fig. 23). It is thus seen that the centrioles come to lie on the periphery in one half of the cell and this half is the one which was originally proximal to the wall of the testis.[1]

The distribution of the centrioles was seen to better advantage in the cell represented by figures 26 and 27. Here they were found entirely confined to the left half of the cell. Those shown in the figures constitute probably less than one-quarter of the total number in the cell, but only those were figured concerning whose identity there could not be the slightest doubt. The same thing may be said of figures 28 and 29 which represent a slightly older cell. The centrioles are seen to have gathered into larger groups and have moved closer together. From each centriole, too, there has grown out a short flagellum. The rays from the centrioles were somewhat obscured on account of the "osmication" of the cell. These, however, are well shown in figures 30, 31, and 32, which represent consecutive sections through a cell of approximately the same age as the preceding one. This cell and others similar to it present the appearance of having polyasters on its one side, while on the other lies the majority of the now vesiculated karyomerites.

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[1] The fact that this half of the cell is the originally proximal one is easily established in the case of such spermatoblasts as have never been forced away from the syncytium. Four facts must be borne in mind: (1) in the young spermatoblasts the position of the centrioles is invariably in the proximal half of the cell; (2) matured spermatoblasts which lie close to the wall of the testis are independent and are not a part of the shrunken syncytium; (3) in such spermatoblasts the centrosome occupies a position distal to the wall of the testis; (4) they undergo their later development in the same position. It is impossible to believe that the centrosome and nucleus of these spermatoblasts have reversed their position during the growth of the cells. On the other hand, in view of the fact that these cells usually lie free except at the point of contact with the syncytium, it will be readily granted that the whole cell has probably revolved. Furthermore, a few instances of spermatoblasts in various stages of growth have been observed, the position of whose centrosomes would indicate a turning of the cell. Therefore, in these spermatoblasts, that portion which appears to be the distal half is really the originally proximal one. After the disappearance of the centrosome in such spermatoblasts as lie close to the wall of the testis, the centrioles come to lie beneath the membrane in the half of the cell directed toward the lumen, that is, in the originally proximal half.

This appearance is not maintained for any length of time, for the centrioles, with the lengthening of their rays, draw together and form a somewhat oval plate just beneath the membrane (fig. 33). The flagella now form a short, thick brush on the outside of the cell. The rays which have now grown very strong, traverse the cell for a short distance as a compact column and then, diverging, they extend to all parts of the cell. A little later the centrioles divide and then the distal halves begin to move across the cell, forming fibers between themselves and the proximal halves. The latter, which are perhaps smaller than the others, flatten themselves against the membrane, each one forming a node at the base of its flagellum.

The mitochondria can now be seen lying above the centrioles and encircling the rays which pass off from them. This irregular ring of granules is shown in cross-section in figures 33 and 35 and in surface view in figure 34. After the dissolution of the nuclear membrane and the dispersal of the karyomerites the mitochondria become obscured for a time, owing to the great difficulty of distinguishing between them and the smaller chromatic elements. They apparently remain in much the same position they occupied before the dissolution of the centrosome. With the vesiculation of the karyomerites they can once again be distinguished, but they now appear much more irregular in outline and size (figs. 28 and 29). Later they form the ring mentioned above.

The spermatoblast has now reached a condition in which it is ready to undergo the growth and differentiations which lead directly to the formation of the adult apyrene spermatozoon. With the completion of the primary growth of the cell, the nucleus has broken down to form a number of small chromatic vesicles. As will be shown later, these are concerned with the secondary growth of the cell, after which their degeneration sets in. By the accumulation of the centrioles at one point of the cell, and their subsequent division, the orientation of the matured apyrene spermatozoon is established; for, as has been pointed out, the antero-posterior axis of the spermatozoon is determined by the direction in which the distal halves of the centrioles traverse the cell. For these reasons the cell may now be spoken of as the apyrene spermatosome.

Before proceeding with the description of the growth and differentiation of the spermatosome, two facts must be considered concerning the spermatoblast: The first has to do with the polarity of the cell and the second with the lack of a strict time correlation between the breaking down of the nucleus and the disappearance of the centrosome, and between the phenomena immediately subsequent to both.

It has already been explained that the orientation of the apyrene spermatozoon is a morphological one, based upon the direction of growth of the bundle of axial fibers. It remains to be pointed out that this direction of growth is determined at the time of the appearance of the first centrosomal structure (the centriole) in the very young spermatoblast (fig. 8) and is parallel with an imaginary line passing through the centriole and the center of the nucleus. This line is the chief axis of the cell and it establishes not only the anterior and posterior poles of the adult spermatozoon, but also the plane of its bilateral symmetry. The centrosome is known to lie in the proximal half of the cell and upon its disappearance the centrioles come to be pretty evenly distributed on the periphery of the same half (figs. 26 and 27). If, in assembling, the centrioles move toward a central point, as there is every reason to believe, then the center of the plate formed by them will lie in the chief axis of the cell. After the division of the centrioles the distal halves move across the cell in a direction vertical to the plate and therefore parallel to the chief axis.

That the chief axis of the cell establishes a bilateral symmetry is shown by comparing figure 1 with figure 2 and figure 3 with figure 4. The first and third of these figures represent two different stages of the apyrene spermatosome, while figures 2 and 4 represent stages almost corresponding to them, but viewed at right angles to them. In the first case, a plane passing vertically through the longitudinal axis of the bundle of axial fibers divides either of the cells into like halves, while in the second case a similar plane divides either of the cells into unequal and dissimilar portions. The same bilaterality is established by the chief axis in the spermatoblast, indeed in very early stages (figs. 12, 13, and 14). Thus it appears that the polarity of the apyrene spermatozoon is determined at a very early stage in its development, at the time when the chief axis is established. The distal side of the spermatoblast marks the anterior end of the spermatozoon and the proximal side the posterior end. The latter may therefore be spoken of as the base of the spermatoblast or spermatosome.

While the dissolution of the nuclear membrane and the disappearance of the centrosome are initiated simultaneously, the phenomena immediately concerned with these two processes and also those subsequent to both do not keep step in different cells. It has already been pointed out that while in one cell (figs. 15 and 16) the centrosome was still perfectly visible after some of the karyomerites had escaped into the cytoplasm, in another (figs. 17 and 18) no traces of the centrosome could be found even before this had occurred. Similarly, in one cell (figs. 19 and 20) all of the centrioles have not yet reached the periphery and the larger karyomerites are fragmenting, while in another (figs. 21 to 23) the centrioles already show their secondary rays, although we are dealing with what is clearly an earlier stage as regards the chromatin. But in a third cell (figs. 24 and 25), and here the greatest variation occurs, we have a condition in which the centrioles have reached the periphery and their secondary rays have not as yet appeared, while the formation of the ultimate karyomerites has already taken place.

The occurrence of variations such as these seems to indicate that the processes concerned with the nucleus have great independence from those concerned with the centrosome and *vice versa*—greater than is known to exist in both normal and abnormal mitosis. In abortive mitoses, such as the so-called oligopyrene spermatocytic divisions of *Paludina*, there is always maintained a constant time correlation between the phenomena which occur in the nucleus and those which occur in the centrosome. An interpretation of the processes described above as being an abortive mitosis of the apyrene spermatoblast of *Strombus* seems to be unwarranted by the facts in the case.

GROWTH AND DIFFERENTIATION OF THE APYRENE SPERMATOSOME.

At first there is very little, if any, growth of the spermatosome; whatever growth there is, takes place slowly and for a long time but little difference in size exists between the spermatosome and the spermatoblast previous to the breaking down of the nucleus. The changes that occur are concerned chiefly with the chromatic vesicles and the centrioles.

By the time the centrioles have divided, the vesiculation of the karyomerites has been completed, or nearly so (figs. 33 and 34). The chromatic vesicles now acquire a very definite position in the cell. They lie in the outer portion of the cell, not far beneath the cell-membrane, and are confined almost entirely to the distal half of the cell. In a section passing through the chief axis of the cell, the chromatic vesicles are seen to lie in a semicircle, the open side of which points towards the base of the cell. This arrangement is shown particularly well in figure 39. At about this time, too, there is a very noticeable swelling of the chromatic vesicles due to the gradual increase in the amount of nuclear sap within them (fig. 41). In many instances an achromatic network is also visible in them (figs. 36, 39, 40, and 41). In a word, the chromatic vesicles now present the appearance of so many small but active secondary nuclei whose reconstruction is not yet completed; and such, indeed, they are considered to be by the writer.

It will be remembered that the secondary rays of the centrioles, at the time of the division of the latter, pass through the ring of mitochondria and then, diverging, they reach to the furthest parts of the cell. There is very little evidence in support of any belief that they attach themselves to the different chromatic vesicles; on the contrary, the ends of many of them can be clearly seen to pass on beyond the vesicles and lie free in the cytoplasm (figs. 33, 34, 35, 36, and 39). After the division of the centrioles, the connection of the rays with the distal halves of the former, or the distal centrioles as they will now be designated, becomes less distinct. In front of the advancing distal centrioles there is formed a dense and homogeneous substance which very frequently obscures those portions of the rays which pass through it (figs. 34 and 35). Into this substance, too, from the distal centrioles, there grow out slender fibrillar processes which become more pronounced as the centrioles advance (figs. 34, 35, 36, and 39). These for a time are probably continuous with the rays, but as the latter become less distinct whatever connection there may be between them is gradually lost (figs. 36 and 39).

As the distal centrioles move away from the base of the cell, there is formed between each pair of daughter centrioles an axial fiber (figs. 34, 35, etc.). These fibers are comparatively stout, more so than the flagella, and they stain very intensely with iron hæmatoxylin. In common with all the other centrosomal structures, they are brought out best in those portions of the tissue where the action of the acetic and osmic acids has not been too strong. In their journey across the cell, spinning out the axial fibers behind them, the distal centrioles come to pass through the ring of mitochondria (figs. 36 and 39). As they approach this ring they undergo a lateral compression in two opposite directions, so that they draw closer together and eventually fuse to form a solid, very flatly oval plate, whose greatest diameter is no less than that of the group of proximal centrioles at the base of the cell. The compression of the distal centrioles with the corresponding flattening of the bundle of axial fibers is shown in figures 36 and 39. It is shown to better advantage, perhaps, in figures 37 and 38, which represent two consecutive cross-sections through a bundle of axial fibers at about the stage shown in figure 39. The first of these cuts the axial fibers near the base of the cell and the second right through the ring of mitochondria; they bring out the difference in contour of the bundle of axial fibers at those two points and give some idea of the enormous number of fibers which constitute the bundle.

In front of the advancing centrosomal plate lies the area of dense material which was mentioned before and which is now becoming more and more evident (figs. 36 and 39). Around the anterior portion of the bundle of axial fibers lie other masses of denser cytoplasm, the inner margins of which are lined by the mitochondria (fig. 38). At this stage, or a little later, in many instances the bundle of axial fibers becomes somewhat twisted and the centrosomal plate is thrown a little out of its horizontal position. Further growth of the axial fibers is accompanied by a further flattening in its anterior portion and a more complete condensation of the centrosomal plate, so that the whole structure, when viewed from the side, has a sharply pointed appearance (fig. 40), while its upper and lower surfaces are as broad anteriorly as they are at the base of the cell (fig. 41). The dense substance in front of the centrosomal plate is pushed on ahead, while the other masses of dense cytoplasm, together with the mitochondria, spread out along the bundle of axial fibers behind the centrosomal plate, forming a sort of sheath around the former. The mitochondria are shown in longitudinal section in figure 40 and in surface view in figure 41.

As is indicated in figure 40, the spreading of the mitochondria and of the dense cytoplasm associated with them results in their separation into two parts, the posterior one of which comes to form a secondary ring around the base of the bundle of axial fibers. As before, this secondary ring is composed of the dense and homogeneous cytoplasm, the inner surface of which is bounded by the mitochondria. A cross-section through the posterior region of the spermatosome shows that the mitochondria of the secondary ring are closely applied to the bundle of axial fibers (fig. 42). The completed separation of the mitochondrial apparatus into an anterior and posterior portion is shown in figure 43. The peculiar structure of the secondary ring is maintained for a long time and the mitochondria connected with it persist in the adult spermatozoon. The mitochondria of the anterior portion, on the other hand, disappear very suddenly, at least no traces of them can be found after the stage represented by figure 43. It will be noticed that here already there has been a diminution of the denser cytoplasm around the anterior end of the bundle of axial fibers; by the time the centrosomal plate has reached the cell membrane practically all of it has disappeared (figs. 44 and 45). While it is possible that the anterior mitochondria may be dissolved *in situ* along with the other substance, two other explanations may be offered for their disappearance: (1) they may move in among the axial fibers and there give rise to occasional mitochondrial granules that are later found scattered throughout the length of the bundle or (2) they may be drawn back to the base of the cell and augment the secondary ring that has been formed there.

By comparing figure 42 with figure 37, it will be seen that after the formation of the secondary mitochondrial ring the posterior portion of the bundle of axial fibers has become more nearly circular, that its diameter is decreased, and that the fibers themselves have drawn closer together. The reduction of the diameter of the bundle is also shown in figures 41 and 42. The process seems to continue proportionately with the gradual growth of the fibers (figs. 44 and 46).

At first the growth of the axial fibers is very much slower than that of the flagella. It will be remembered that the flagella begin to grow out before the centrioles have divided. By the time the distal centrioles have traversed one-third the distance across the cell, the flagella have reached their maximum growth and are more than twice as long as the axial fibers (figs. 33, 34, 35, 36, and 39). From this point on they gradually become shorter and more ragged in appearance, eventually fusing at a very late stage to form the posterior tip of the spermatozoon (figs. 40, 41, 43–49, 63, and 64). This shortening of the flagella is probably due to their partial retraction into the cell, although their ragged appearance would suggest that they have been worn down by attrition with other free cells.

v. Brunn ('84) has described a similar action on the part of the flagella for the oligopyrene spermatozoa of *Paludina*. His observations were confirmed by Meves ('03), who explains the phenomenon on the ground that the flagella are the free ends of the axial fibers, each one passing through a ring formed by one of the proximal centrioles upon the cell-membrane. The shortening of the flagella is caused by the downward growth of the cell substance around and between them, so that, eventually, a portion of each flagellum comes to lie within the body of the cell. The facts that have been observed in the case of *Strombus* do not support this view. In the first place, there is not the slightest morphological evidence that the proximal centrioles form rings; they clearly form nodes just beneath the membrane and each one lies at the base of a flagellum. Then again, the flagella have begun to shorten before there has been any appreciable lengthening of the spermatosome in either direction; if any has occurred, it is certainly not sufficient to account for the very noticeable shortening of the flagella that has taken place.

It remains to be pointed out that there exists a striking similarity between the apyrene spermatosome (as regards its centrosomal structure at the time of the early growth of the axial fibers) and the well-known ciliated epithelial cells of the Mollusca. In figure 35, for example, the proximal centrioles can well be compared with the so-called basal granules of the ciliated epithelial cells and the distal centrioles with the inner granules. The slender fibers growing out from the distal centrioles are analogous to the fibers which extend across the epithelial cell. With the progressive growth of the axial fibers across the spermatosome the resemblance disappears to a great extent. While a strict homology between the two structures can not be maintained, we have here a strong, if indirect, support for the Lenhossék-Henneguy theory of the centrosomal origin of the basal granules found in a ciliated cell.

Several changes occur in the spermatosome about the time that the distal plate of centrioles reaches the cell-membrane. At this time or perhaps a little earlier, the chromatic vesicles begin gradually to lose their sap, so that they decrease in size and the chromatic granules come closer together (figs. 43–45). As the process continues the chromatin gathers on one side of the vesicle which is now becoming more and more reduced in size (figs. 46, 47, 48, and 49). Soon the membrane disappears, either by being dissolved or by the total loss of the sap, so that the whole structure shrinks into a solid mass. In either case, what were formerly active chromatic vesicles are now reduced to degenerating lumps of chromatin (fig. 63); eventually, all of these are dissolved in the cytoplasm and disappear, leaving no traces in the adult spermatozoon. The whole process of the degeneration of the vesicles can be followed through in the living cells (figs. 1–6).

The direct effect of the degeneration of the chromatic vesicles is found in the appearance at this time of the secreted albuminous bodies. Just as in some epithelial cells where the formation of the secreted granules is accompanied by the degeneration of the nucleus, so here the processes leading to the eventual disappearance of the chromatic vesicles go hand in hand with the secretion of the albuminous bodies. A similar correlation between the degeneration of the nucleus and the secretion of the albuminous bodies has been described for the so-called nurse-cells of *Littorina* (Reinke, '12). In *Strombus* the albuminous bodies appear first in the anterior portion of the spermatosome; a few can always be found around the advancing end of the bundle of axial fibers by the time it has grown across the cell. In later stages they are formed in increasing numbers and gradually come to fill up first the middle and then finally the posterior portion of the spermatosome (figs. 48, 49, 63, and 64; also figs. 1–6). By the time that all the chromatin has disappeared from the cell, practically all of the albuminous bodies have been formed (fig. 64).

The formation of the albuminous bodies takes place in very much the same way as Kuschakewitsch ('11) has described for the apyrene spermatozoon of *Vermetus* and which has been briefly reviewed on page 204. In *Strombus* there is not a general differentiation of the cytoplasm into a large number of thick-walled chambers. Instead, there is a continual formation of occasional vacuoles throughout the cell, those that are first formed filling up rapidly with the secreted albumen before the later ones have appeared. For this reason one never sees more than three or four vacuoles in any one given cell (figs. 43, 47, and 49). The extreme vacuolization shown in figure 46 is due to imperfect fixation and is not a natural condition. The process of the secretion of the albuminous bodies can be followed most readily in the posterior region of the spermatosome.

In a vacuole such as one of those shown in figure 49, a matrix is laid down composed of a substance which has a marked affinity for nuclear stains. The appearance of this matrix differs to some extent with the degree of "osmication" which the cell has undergone. In the inner portions of the tissue it is seen to be composed of a number of very irregular, darkly staining masses (fig. 52), while in the outer portion it appears to have the form of a network (fig. 63). It must be borne in mind that the magnification in figures 63–65 is less than in the preceding figures. Between these two extremes there stands a condition in which the network is made up of granules. Around this substance a clearer, homogeneous material is secreted, which gradually fills up the vacuole and effaces the matrix. In many instances narrow strands can be seen connecting this substance (an albumen?) with the surrounding cytoplasm (figs. 46, 49, and 54).

When the vacuole has been completely filled, a membrane is formed around the secretion, the body assuming a spherical shape. The bodies now give up their stain (iron hæmotoxylin) very readily; in the places where the spermatosome has been strongly "osmicated," they either appear unstained and yellow, or else they show the characteristic "Spiegelfärbung" of yolk granules. As has been stated, their change to the hexagonal shape seen in the adult condition is due to the pressure they exert upon one another after they have completely filled the spermatosome and the latter has undergone its final elongation and constriction.

By far the most striking changes that begin to take place at the time when the distal centrioles reach the cell membrane (figs. 44 and 45) are those which result from the continued growth of the axial fibers, viz., the formation of the undulating membrane and the resulting changes in the size and shape of the spermatosome. Having come to extend entirely across the cell, the bundle of axial fibers, by their continued growth, begins to protrude beyond the original boundary of the cell, causing the spermatosome to acquire a flask-shaped appearance (figs. 3 and 46). In the "neck" of the spermatosome, those yolk bodies which were the first to be formed come to lie between the broad surfaces of the bundle of axial fibers and the cell-membrane; the edges of the bundle, on the other hand, lie in close contact with the cell-membrane (figs. 3, 4, and 48).

At this stage, in the living spermatosome, there is always to be seen a very noticeable protuberance on one side of the brush formed by the flagella. This structure, although it is only a transitory one, is very constant in the living cells. The first indication of it is shown in figure 2, where it appears as a swelling at the base of the cell. Later it becomes very much longer and flattened in one axis (figs. 3 and 4) and appears finally to be withdrawn into the cell-body (fig. 5). The only trace of this protuberance that could be found in sectioned material is shown in figure 40. The point at which this protuberance occurs must mark the weakest part of the cell membrane; this is shown by the changes that occur when degeneration sets in. After a spermatosome in the stage represented by figures 3 or 4 has been in an artificial medium for two or three hours, the protuberance begins to become very much distended. This is followed by a shifting, to that quarter, of all the free contents of the cell, with the result that the bundle of axial fibers, instead of passing through the cytoplasmic body, lies tangentially to it and is surrounded by the cell membrane alone. This protuberance may be interpreted as a precocious lengthening of the cell in a posterior direction, the completion of which is prevented by the actual attachment of the fibers to the cell-wall. As will be shown presently, such a lengthening does take place at a very much later stage, but this time it is accomplished through the direct agency of the axial fibers themselves.

After a spermatosome has reached the stage represented by figure 46, a longitudinal split occurs in the bundle of axial fibers, dividing it into two approximately equal halves (fig. 47). This is caused by the continued growth of the axial fibers without a corresponding lengthening of the cell. Growth of the spermatosome does take place, to be sure, resulting in its becoming longer and narrower, but this growth is not commensurate with that of the axial fibers, nor is it accompanied by an increase in the volume of the cell. If these facts are borne in mind, then the following steps in the formation of the undulating membranes will be easily understood.

After the split occurs in the bundle of axial fibers, the two halves, or the secondary bundles, as they may now be called, move apart from each other, and gradually approach the cell-membrane from opposite sides. A lateral view of one of the secondary bundles, shortly after this movement has commenced, is shown in figure 48. By comparing figures 47 and 48, it will be seen that the albuminous bodies already formed are so placed that they do not obstruct the path of the fibers; those that eventually come to lie in the middle and posterior portion of the spermatosome are not secreted until after the fibers have reached the membrane on each side of the cell (figs. 49 and 63). As the secondary bundles separate, the individual fibers composing them fold back upon each other, so that several twists are formed in the bundles (figs. 49 and 63). Owing to the shape of the spermatosome, the secondary bundles reach the membrane in the anterior part of the cell long before they do so in the posterior region. Correspondingly, the differentiation of these structures to form the undulating membranes takes place progressively from the anterior to the posterior end of the spermatosome. Consequently, a series of cross-sections passing in the opposite direction through a spermatosome in the stage represented by figure 49 should show all the changes that take place both before and after the secondary bundles reach the surface.

Such a series is represented by figures 50 to 58. In figure 51 it is seen that the secondary bundles, in moving apart, have broken the ring of mitochondria which was mentioned before as having been formed around the base of the primary bundle. Afterwards, the mitochondria come to lie between the secondary bundles, while the dense cytoplasm which has been associated with them is gradually dissolved (figs. 49 and 63). It will be noticed that posteriorly the secondary bundles are wider than they are anteriorly. As they approach the surface the fibers draw closer and closer together; as a result, the bundles stain more and more deeply and it becomes increasingly difficult to distinguish the individual fibers. It can be seen, however, and very clearly too, that there is a modification taking place in that those fibers which lie nearest the cell-membrane tend to draw slightly away from the others and to fuse with one another (figs. 52 and 53). By the time the bundles have reached the surface, the fusion of these outer fibers has been completed and they are so closely applied to the cell-membrane that the latter becomes indistinguishable from them (fig. 54). The next figures show successive stages of the process by which the secondary bundles push out the cell-membrane and come to extend laterally beyond the body of the cell. Figure 58 represents a section passing just behind the centrosomal plate and shows that the fibers have arranged themselves into definite rows, a fact also indicated in figures 56 and 57. As will be shown presently, this arrangement of the fibers is necessary for the complete formation of the undulating membranes.

At a somewhat later stage, after the secondary bundles have reached the cell-membrane throughout their entire length, the myoneme-like striations are formed. This process is shown in figures 59 and 60. Some of the fibers which lie next to the cell-membrane now separate from their fellows and begin to move out across the surface of the cell just beneath the membrane. From this it would seem that there is not an actual fusion of the exterior fibers of the secondary bundles, but that they lie in very close contact with each other. It is probable, too, that the exterior fibers do not participate in the twisting of the secondary bundles spoken of above. Since the fibers are attached at both ends, the twisting of the secondary bundles must take place at their middle portions and doubtless proceeds in one direction and then in the other; it is simply an accompaniment of the separation of the secondary bundles and after these have reached the cell-membrane such twists as have taken place are apparently undone. At all events, as the secondary bundles begin to push out beyond the cell-body, a rearrangement of the inner fibers occurs in that they form a number of horizontal layers (figs. 56, 57, 58, and 60). Some of the inner fibers, apparently, crowd into the layer of exterior ones, taking the place of those that have moved away, while the others continue to form a decreasing number of horizontal layers. The result is that the bundles become wider and flatter. This is shown in cross-section in figures 61 and 62 and in surface view in figure 65. The first of these figures is an oblique section through the anterior region of a spermatosome at about the stage represented by figure 64. The section represents a cross-section just behind the centrosomal plate of another spermatosome of the same age. Figure 65 is a longitudinal section through a spermatosome which was almost identical with the one represented by figure 64. In this last figure the myoneme-like striations are also shown in surface view. As is shown here, the same force (that is, the continued growth of the axial fibers without a fully compensating increase in the length of the cell-body) which causes the secondary bundles to reach the surface of the cell and then to extend beyond it, eventually causes several folds to occur throughout their length. This is also indicated in figure 61.

After the secondary bundles have reached the cell-membrane, the space which has been vacated by them is filled with a substance whose consistency is greater than that of the cytoplasm surrounding the albuminous bodies (fig. 53). Indeed, there seems to be a general flowing of the denser, more granular ctyoplasm to the center of the cell, leaving the homogeneous enchylema to fill the spaces between the albuminous bodies (figs. 59 and 60). Eventually this denser part of the cytoplasm becomes differentiated in such a way that it forms a fibrillar core passing down through the center of the cell (fig. 64).

The changes in the shape of the spermatosome, as a result of the splitting of the bundle of axial fibers and the subsequent growth of these fibers themselves, are shown in figures 47, 49, 63, and 64. At first, accompanying the spreading of the secondary bundles to the surface of the cell, there is a slight elongation of the spermatosome in an anterior direction (fig. 49). This is continued until the secondary bundles begin to protrude beyond the cell-body, when it practically ceases (fig. 63). It will be noticed that the centrosomal plate has been pushed right up to the very anterior tip of the spermatosome. The further growth of the fibers results next in the lateral extension of the secondary bundles beyond the cell-body throughout their entire length and then in the elongation of the spermatosome in a posterior direction (fig. 64). This fact is evidenced not only by the change in the contour of the posterior end of the spermatosome, but also by the redistribution of the mitochondria. The base of the cell has become pointed and the flagella have fused to form the extreme end of the tail-piece. About this time, too, the folds that were mentioned before are formed in the secondary bundles.

A spermatosome which is in the stage represented by figures 64 and 65 is now ready to undergo the final change which transforms it into the fully formed apyrene spermatozoon (fig. 6). It has been completely filled with the albuminous bodies and all the chromatin has been dissolved in the cytoplasm; the secondary bundles have come to form folded ridges on both sides of the cell, already indicating the final shape of the undulating membranes. The length of the spermatosome, however, exclusive of the flagella is only about 70 micra. A general lengthening and constriction of the cell now take place which result in its becoming spindle-shaped, with an increase of from 15 to 20 micra in its length. At the same time the folds of the secondary bundles are somewhat straightened out and they themselves become much broader and flatter, assuming the shape and appearance of typical undulating membranes. The fibers are now probably arranged into two outer layers where they are in close juxtaposition to one another, if not actually fused, and a single inner layer where they are more widely separated from each other. The base of the cell is drawn out narrowly to a point, ending with the fused flagella. It is quite possible that the final change in the shape of the cell is to be attributed to the continued growth of the fibers.

DISCUSSION.

There are two outstanding differences between the development of the apyrene spermatozoa of *Strombus* and that of the oligopyrene spermatozoa of *Paludina*. The first of these is in regard to their origin. It has been shown by Meves ('03) that the oligopyrene spermatozoa of *Paludina* arise from the spermatogonia, certain ones of which undergo an unusual growth and become the oligopyrene spermatocytes. In *Strombus* the apyrene spermatozoa arise from certain cells which are different from the spermatogonia and which have been called the apyrene spermatoblasts. Secondly, in their later development, the apyrene spermatozoa of *Strombus* do not show nearly as close a parallelism to the development of the eupyrene as do the oligopyrene spermatozoa of *Paludina*. In both forms the development of the bundle of axial fibers in the atypical spermatozoon can be closely homologized with the growth of the axial fiber in the true spermatozoon, but the two divisions of the oligopyrene spermatocyte which take place in *Paludina* are entirely lacking in the apyrene spermatoblast of *Strombus*. Again, in the adult oligopyrene spermatozoon of *Paludina* the equivalent of one chromosome is retained and forms its nucleus, while the apyrene spermatozoon of *Strombus* contains no chromatin at all. Finally, it might be pointed out that the oligopyrene spermatozoon of *Paludina* shows a much closer approximation to the form of the eupyrene than does the apyrene spermatozoon of *Strombus*.

On the other hand, there exist certain fundamental similarities of development and structure between the atypical spermatozoa of *Strombus* and *Paludina* and indeed of other forms in which they have been described. The first of these is the relatively immense growth of the cell which ultimately gives rise to the atypical spermatozoon. Second, the history of the centrosome and the development of the axial fibers of the atypical spermatozoon present close parallels in *Strombus* and *Paludina*. The centrosome fragments and gives rise to a number of centrioles; the division of these with the subsequent separation of the daughter centrioles results in the formation of the bundle of axial fibers. In *Strombus* the differentiation is carried further, in that the bundle of axial fibers splits and the two halves move laterally to the surface of the cell and there form the two undulating membranes. Very similar phenomena have been described by Stephan ('03*b*) and by Lams ('09) for *Murex* and by Kuschakewitsch ('11 and '13) for *Vermetus*. In fact, in every form in which the development of the atypical spermatozoa has been described we find the differentiation of the centrosomal element into axial fibers.

Perhaps the most generally constant phenomenon in the development of the atypical spermatozoa of different forms is the fate of the chromatin. In *Paludina*, according to Meves ('03), one chromosome is retained and forms the nucleus of the adult oligopyrene spermatozoon, while all the rest of the chromatin degenerates. A similar condition is held by Lams ('09) to be the case in *Murex*, although Stephan ('03*b*) had previously reported that in this form all of the chromatin degenerates. In all other forms in which the matter has been investigated, the nucleus breaks down and an atypical spermatozoon is developed which contains no chromatin. Another characteristic held in common by the atypical spermatozoa of certain forms is the fact that the products of metabolism are stored in the cell. Thus, we find large secreted bodies in the atypical spermatozoa of *Strombus*, *Pteroceras* (Brock, '87), and *Vermetus* (Kuschakewitsch, '11 and '13). It is probable, too, that the mitochondrial apparatus, so beautifully described and figured by Retzius ('05), in the oligopyrene spermatozoa of *Paludina* is of the same nature, as well as the refractive bodies described in the atypical spermatozoa of *Cypræa* by Brock ('87), of *Murex* by Koehler ('88), and other writers and the vacuoles in the apyrene spermatozoa of *Conus* described by Schiemenz ('96) and Kuschakewitsch ('11 and '13).

Both Stephan ('03*a*) and Kuschakewitsch ('11) have suggested the possibility of arranging the atypical spermatozoa of different forms into a gradated series based upon differences in their development and structure and representing further and further retrogressions from the typical spermatozoa. At the head of such a list would be placed *Paludina* and *Murex* in the order named. Next would come *Conus*, for here Kuschakewitsch ('13) has recently found that one division normally occurs in the development of the apyrene spermatozoon. After these there might follow in succession *Cerithium*, *Vermetus*, and *Strombus*. The list can not safely be extended further than this, since we are not sufficiently acquainted with the phenomena which take place in other forms; and even as it stands the list is full of contradictions. Thus, for instance, *Cerithium* should perhaps precede *Conus*, since in the former one chromosome is retained in the apyrene spermatozoon for a longer time than the others (Stephan, '03*a*), while in *Conus* all the chromatin of the apyrene spermatocyte degenerates simultaneously. Again, when considered from the standpoint of the differentiations of the centrosome and the development of a motor apparatus, *Conus* should be placed at the end of the list, for here the apyrene spermatozoa are totally immotile. *Vermetus* has been placed before *Strombus* because of the formation of chromosomes and of an abortive spindle in the development of the apyrene spermatozoa of the former; but the order could well be reversed when it is borne in mind that, in the development of the bundle of axial fibers of the atypical spermatozoon, *Strombus* resembles *Paludina* much more than does *Vermetus*. The truth of the matter is that we do not have sufficient data upon which to formulate such a series. In the writer's opinion, the evidence is not clear enough for us to draw correct inferences as to whether or not we are dealing with a series of retrogressions from the atypical spermatozoa.

While it has been shown that the atypical spermatozoa, in all those forms in which they have been studied, show certain fundamental similarities, each one also has certain differences peculiar to itself. One of the greatest of these differences, perhaps, is that in regard to the origin of the apyrene spermatozoa of *Strombus*. Nevertheless, in spite of such differences, it is the writer's opinion that they are all essentially the same and are derived from similar elements of the testis—that is, they are modifications of the accessory cells of the testis, just as are the nurse-cells in the testis of *Littorina*. It is suggested that the explanation of the differences between them is to be found in the manner in which the differentiation of the primitive cells of the sexual gland takes place into the various elements of the testis. So, in the Pulmonates there is a sharp differentiation of the primitive sex-cells into the true sex-cells and into the accessory cells, the basal nuclei with their surrounding syncytium. Here the latter never become differentiated into independent cells, although they undergo changes in their size and shape (Platner, '85). Among the Prosobranchs, in *Littorina*, the writer has never observed the same tremendous swelling of the basal nuclei as may be seen in the ovotestis of *Planorbis*, but, instead, certain of them give rise directly to the nurse-cells to which the spermatozoa are attached. In *Strombus* those elements which give rise to the atypical spermatozoa are differentiated from the true sex-cells along with the basal nuclei. The latter never give rise to nurse-cells of any kind. In forms such as *Paludina* and *Murex*, the elements which give rise to the atypical spermatozoa are not differentiated from the true sexual elements along with the basal nuclei, but only after the spermatogonia have been formed. In *Paludina* the formation of nurse-cells has never been observed by the writer nor have they been described by Meves, but the basal nuclei undergo changes which are very similar to those which occur in the ovotestis of

Planorbis. In *Vermetus* and in *Conus*, too, according to Kuschakewitsch ('13), the atypical spermatozoa are not differentiated until after the formation of the spermatogonia. But in both these forms nurse-cells are also developed and apparently from the basal nuclei; whether or not their development is the same as that of the nurse-cells in *Littorina* is not known by the writer. In all these forms there is a period in the development of the testis when the true sex-cells are undifferentiated from the accessory elements. It may be that certain differences in the plasma of the primitive cells lead to differences in the manner of differentiation of the accessory elements and these in turn lead to the production of adult structures which differ from each other to a greater or less degree, but all of which show certain fundamental resemblances.

Besides the characteristics held in common between the atypical spermatozoa of *Strombus* and those of other forms, the former show certain resemblances to the nurse-cells in the testis of *Vermetus* and of *Littorina*. While there are no large cells in the testis of *Strombus* which could possibly be taken for anything except the apyrene spermatoblasts, there exist a striking resemblance between certain of the developmental stages of these and some of the figures given by Kuschakewitsch ('13) for the nurse-cells in the testis of *Vermetus* (figs. 104 and 105, Taf. XXIV, and figs. 169 and 170, Taf. XXV, Arch. f. Zellforsch., Bd. X). The writer has shown that nurse-cells are developed in the testis of various species of *Littorina* which, when they have reached their maximum size, resemble the oligopyrene spermatocytes of *Paludina*. Later on, the cells become filled with large secreted bodies, while their nuclei gradually degenerate. In the final stages the latter may still be seen in a very shrunken, degenerated condition lying just beneath the cell-membrane. It is in connection with the formation of these secreted bodies and the corresponding changes in the nucleus that the apyrene spermatozoa of *Strombus* resemble the nurse-cells of *Littorina*. It will be remembered that in the apyrene spermatoblasts, after the nucleus breaks down, chromatic vesicles are formed which gradually increase in size until the formation of the albuminous bodies is begun. From this point on, with the continued formation of the albuminous bodies, they gradually degenerate and are finally all absorbed in the cytoplasm.

A comparison of the processes which occur on these cells and those which take place in normal secretory cells lead to some interesting considerations. Mathews ('99) has shown that secretory activity or, to use his term, hylogenesis, is accompanied in various epithelial cells by certain changes in the nucleus, in that the latter becomes irregular in outline and is displaced towards the base of the cell. In these cells the process is cyclic; after the cell has been exhausted the nucleus returns to its normal condition and the activity begins over again. The changes which the nurse-cells of *Littorina* undergo at the commencement of their secretory activity are very similar to those which take place in secretory epithelial cells. In the former case, however, the process is not repeated and in every instance it is accompanied by the gradual degeneration of the nucleus. It seems to be not unlikely that these two facts stand in a causal relation to each other.

The same condition, that is, the unrepeated secretory activity of the cell accompanied by the degeneration of the nucleus, holds true in the case of the apyrene spermatozoa of *Strombus* as well as in the nurse-cells of *Littorina.* But here the question might well be asked, why does not the nucleus degenerate directly, instead of undergoing the complicated history that has been described? The primary function of the nucleus is concerned first with the growth of the spermatoblast and then with the secretion of the albuminous bodies, and possibly its presence is essential for the development of the great centrosome with its many centrioles. The differentiations which occur in connection with the centrioles lead to the formation of the undulating membranes and they are initiated by the disappearance of the centrosome. It is believed that the impulse, whatever it may be, which is responsible for the disappearance of the centrosome causes also the dissolution of the nuclear membrane. That impulse may be the same as that which causes a cell to divide mitotically, but, as has been shown, there is no real evidence to support such an assumption. Stated a little differently, when considered in regard to the subsequent development of the apyrene spermatosome, it is the change that occurs to the centrosome that is of prime importance at this time and that which occurs to the nucleus is incidental to it.

Were this not so, we should expect either that the chromatic fragments would degenerate without first becoming vesiculated and the secretion of the albuminous bodies be commenced at the same time, as is the case in the apyrene spermatozoa of *Vermetus,*[1] or else that the nucleus would remain intact until the secretory activity of the cell is begun and then degenerate as a whole, which is the case in the nurse-cells of *Littorina.* Instead, the karyomerites become vesiculated and form a great number of secondary nuclei which increase in size until the first albuminous bodies are formed, when they begin to degenerate. Moreover, if the secretory activity of the cell is enhanced by an increase in the surface contact between the nucleus and the cytoplasm, then the formation of the secondary nuclei is of great advantage.

The conditions observed in the seminal receptacle and uterus of *Strombus bituberculatus* and *S. gigas* after copulation and at a time before oviposition has commenced, and indeed before the ova are fully matured, throw a certain amount of light upon the nature of their function. The fact that eupyrene spermatozoa are found in those parts at such a time indicates that they, or at least some of them, must remain there for a protracted period—that is, until and during the time of oviposition. The greater part of that time is no doubt spent in the seminal receptacle, where they can and do receive an independent supply of nourishment. But, judging from the fact that in nearly every case examined they have also been found in the uterus along with the apyrene spermatozoa, it must take a long time for them to move into the seminal receptacle. In the uterus a separation of the two kinds of spermatozoa occurs, the eupyrene being retained in good condition while the apyrene may be seen in all conditions of katabolic changes leading to the total exhaustion of the secreted bodies. It may be that the apyrene spermatozoa serve to nourish the eupyrene until the latter are stored in the seminal receptacle, where they may obtain an adequate and independent food supply. Whether this is so or not, it is believed that the apyrene spermatozoa of *Strombus* subserve their purpose in the uterus and possibly also in the vagina and bursa seminalis immediately after copulation and that their function is connected with their breaking down.

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[1] In this connection Kuschakewitsch ('13) has made the suggestion that in *Vermetus* the retention of the chromatic elements as such almost until the end of spermatogenesis is to be explained on the grounds that the chromatin participates in the formation of the albuminous bodies in the protoplasm of the apyrene spermatozoon.

There are two other ways of explaining the behavior of the apyrene spermatozoa after copulation has taken place. The first of these was suggested to the writer by Professor Conklin and is to the effect that the apyrene spermatozoa in breaking down may liberate a substance which stimulates the eupyrene spermatozoa or the eggs or both during fertilization. The possibility of their being an aid in the final disposition of the eupyrene spermatozoa was indicated by a series of experiments recently performed by the writer.[1] It may be that the substance liberated by the apyrene spermatozoa as a result of the changes which they undergo causes the eupyrene spermatozoa to withdraw from them and eventually to enter the seminal receptacle.

The fact that the apyrene spermatozoa of *Strombus* break down after copulation and are discarded, while the eupyrene are retained and kept in a healthy condition, together with the results obtained by Kuschakewitsch ('10) in his study of the fertilization of the eggs in *Aporrhais pes pelicani*—these facts, in the writer's opinion, preclude the view that the atypical spermatozoa may participate as such in the fertilization of the egg.

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[1] While at the Tortugas Laboratory during June 1913, cultures of the contents of the sperm-duct of *Strombus gigas* were kept alive and observed under varying conditions. The spermatozoa lived longest in cultures made from sterilized sea-water, in some cases as long as 72 hours. The length of life of the spermatozoa apparently depended upon the extent to which the cultures had been kept free from bacterial infection. In such cultures, there was always a separation of the two kinds of spermatozoa, the eupyrene remaining active for a long time after the apyrene had ceased their movements. In very many instances a clear drop of some substance could be seen attached to the side of an apyrene spermatozoon whose contents had partially or completely broken down. Very striking results were obtained in a few cultures in which a small piece of decaying tissue was included in order to test the reaction of the two kinds of spermatozoa to CO_2. In every case the eupyrene spermatozoa moved further and further away from the source of the CO_2 until they reached the edges of the cover glass. The apyrene spermatozoa, on the other hand, were not greatly stimulated except in one instance, where they gave a distinct, positive reaction, gathering around the pieces of decaying tissue. One effect of CO_2 upon the apyrene spermatozoa was to cause a comparatively rapid and complete breakdown of their contents. Unfortunately, for lack of time, these experiments could not be repeated under conditions of greater accuracy.

SUMMARY.

1. The apyrene spermatozoa of *Strombus*, in the adult condition, are devoid of any nuclear material. The body of the spermatozoon is spindle-shaped and is filled with large hexagonal albuminous bodies. Locomotion is brought about by means of the undulations of two broad membranes attached to each side of the body.

2. Together with the eupyrene spermatozoa, they are found in the uterus of the female, where they undergo certain katabolic changes resulting in the total exhaustion of the albuminous bodies. They are never found in the seminal receptacle, even when the latter is crowded with eupyrene spermatozoa.

3. The differentiation between the eupyrene and the apyrene spermatozoa can be traced back further than the growth period. In fact, apyrene spermatozoa develop from cells which can be distinguished from the spermatogonia of the first generation and which have been named the apyrene spermatoblasts.

4. The apyrene spermatoblasts are believed to arise from the basal nuclei. Confirmation of this phenomenon in other related forms is required before it can be considered an established fact.

5. The very early appearance of a centriole in the apyrene spermatoblast establishes the polarity of the apyrene spermatozoon.

6. The growth of the apyrene spermatoblast is marked by a great increase in the volume of the cytoplasm and nucleus and by the development, from the original centriole, of a very large centrosome containing many secondary centrioles at its periphery. No division of any kind takes place during the development of the spermatoblast.

7. After the apyrene spermatoblast has acquired its full growth, the dissolution of the nuclear membrane and the disappearance of the centrosome take place simultaneously. The chromatic fragments (karyomerites) are scattered throughout the distal half of the cell and the centrioles become grouped beneath the membrane at the base of the cell. From each of the latter a flagellum grows out. The cell may now be spoken of as the apyrene spermatosome, for the ensuing changes give rise directly to the adult apyrene spermatozoon without any appreciable increase in its volume.

8. The centrioles divide and the distal halves move across the cell in the direction of the chief axis, forming a bundle of axial fibers between themselves and the proximal halves. At the same time the karyomerites become vesiculated and remain active for a time, gradually becoming larger.

9. The mitochondria which have been formed around the centrosome during the growth period now constitute the inner margin of a ring of dense cytoplasm which lies around the bundle of axial fibers at its base.

10. When the bundle of axial fibers has come to extend entirely across the cell, the secretion of the albuminous bodies is begun in the anterior portion of the spermatosome. This process continues hand in hand with the degeneration of the chromatic vesicles, until, successively, the middle and

posterior portions of the cell have been completely filled with them and all of the chromatic vesicles have disappeared.

11. In the meantime, the bundle of axial fibers continue to grow until the cell has been elongated in an anterior direction, along its chief axis, by about one-third its original diameter. Afterwards, the continued growth of the axial fibers is not accompanied by a fully compensating increase in the length of the cell; the changes which ensue are all the direct result of this fact.

12. A longitudinal split occurs in the bundle of axial fibers, dividing it into two equal halves, each of which moves in an opposite direction towards the surface of the cell. In doing so they rupture the ring of dense cytoplasm which lie around the base of the bundle. This substance gradually disappears, but the mitochondria associated with it persist in the adult apyrene spermatozoon.

13. Having reached the surface of the cell, the secondary bundles of axial fibers now come to extend laterally beyond it. The further growth of the fibers causes a number of folds to occur throughout the length of the bundles. Finally a general elongation and constriction of the cell occur, with the result that the body of the spermatosome acquires the spindle-like shape of that of the adult apyrene spermatosome and the secondary bundles on both sides of it become straighter and more flattened, forming the undulating membranes.

14. In the writer's opinion the apyrene spermatozoa of *Strombus* subserve their purpose after copulation has taken place and their function is connected with the katabolic changes which they undergo. Their behavior may be explained on the grounds of the following three suggested possibilities: (*a*) They may serve as nurse-cells to the eupyrene spermatozoa after copulation and before the latter reach the seminal receptacle; (*b*) they may, by the liberation of some substance, stimulate the eupyrene spermatozoa or the eggs or both during fertilization; or, by the liberation of some substance to which the eupyrene spermatozoa are negatively chemotactic, they may act as an aid in the final disposition of the latter.

15. The conditions met with in the uterus and seminal receptacle of the female after copulation has taken place and before oviposition has commenced show that the apyrene spermatozoa do not participate as such in the fertilization of the egg.

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EXPLANATION OF FIGURES.

All figures were drawn at table height with the aid of a camera lucida. With the exception of those on plate 1 and figs. 63–65, all figures were drawn with the use of the Zeiss 1.5 mm. apochromatic objective and the 12 compensating ocular; the figures on plate 1 were drawn with the use of the Zeiss 2 mm. apochromatic objective (N.A. 1.30) and the 6 compensating ocular and were then enlarged to 1825 diameters, while figs. 63–65 were drawn with the use of the Zeiss 1.5 mm. apochromatic objective and the 8 compensating ocular. In studying the cells the Zeiss 1.5 mm. apochromatic objective as well as the 2 mm. (N.A. 1.40) were used in combination with the 8 compensating ocular. Figs. 8–65 were drawn from sections, 5 micra thick, of material fixed in Flemming's fluid (stronger solution) and stained with iron hæmatoxylin followed by gentian violet and erythrosin.

PLATE 1.

(Initial magnification 1825 diameters, not reduced.)

1 to 5. Three progressive stages in the development of the apyrene spermatosome, drawn from living material. Figs. 1 and 2 show corresponding stages viewed in planes at right angles to each other; figs. 3 and 4 are similar views of a later stage.

6. Adult apyrene spermatozoon, drawn from a specimen fixed with osmic vapor and stained with iron hæmatoxylin.

7. Adult eupyrene spermatozoon, drawn from a specimen fixed with osmic vapor and stained with iron hæmatoxylin.

PLATE 2.

(Initial magnification 3450 diameters, reduced one-third.)

8 to 14. Various stages in the growth of the apyrene spermatoblast from the earliest appearance of the centrosomal structure until a time just before it disappears and the nuclear membrane is dissolved.

15 and 16. Two consecutive sections through a single cell showing the initiation of the changes which occur to the nucleus and the centrosome. The mitochondria are shown lying around the centrosome (fig. 15).

17 and 18. Two consecutive sections through a single spermatoblast in the same stage as the preceding one. Note that the centrosome has disappeared.

19 and 20. Two consecutive sections through a single spermatoblast showing the scattering of the chromatic masses after the dissolution of the nuclear membrane.

PLATE 3.

(Initial magnification 3450 diameters, reduced one-third.)

21, 22, and 23. Three consecutive sections through a single spermatoblast showing the appearance of groups of centrioles at the periphery of the cell.

24 and 25. Two consecutive sections through a single spermatoblast showing the completed fragmentation of the chromatic masses to form the ultimate karyomerites.

26 and 27. Two consecutive sections through a single spermatoblast showing the appearance of the centrioles on the periphery of the originally proximal half of the spermatoblast and the vesiculation of the karyomerites.

28 and 29. Two consecutive sections through a single spermatoblast, showing the grouping of the centrioles and the growth of the flagella.

30, 31, and 32. Three consecutive sections through a single spermatoblast of the same age as the preceding one, showing the rays which extend from the centrioles into the interior of the cell.

PLATE 4.

(Initial magnification 3450 diameters, reduced one-third.)

33. A section through a very young spermatosome. The centrioles have gathered at the base of the cell but have not yet divided.

34. A section through a spermatosome in the stage in which the centrioles are dividing.

35. A section through a spermatosome after the division of the centrioles showing the manner in which the axial fibers are formed.

36 and 39. Sections through two spermatosomes showing consecutive stages in the growth of the axial fibers.

37 and 38. Two consecutive sections through the bundle of axial fibers of a spermatosome in a stage corresponding to that shown in figure 39. Note the almost round base and the flattened anterior portion of the bundle and the mitochondrial apparatus surrounding the latter.

40 and 41. Sections through two spermatosomes of approximately the same age; the sections pass in planes which are at right angles to each other. Figure 41 gives a surface view of the bundle of axial fibers and the mitochondria surrounding it; note the increase in the size of the chromatic vesicles shown here.

42. A cross-section through the posterior region of a spermatosome which is a little older than the preceding ones. Note how closely the mitochondria are applied to the bundle of axial fibers.

PLATE 5.

(Initial magnification 3450 diameters, reduced one-third.)

43. A section through a spermatosome showing the further growth of the bundle of axial fibers.

44 and 45. Sections through two spermatosomes showing the bundle of axial fibers extending across the cell and the early formation of the secreted bodies; the sections pass in planes which are at right angles to each other.

46. A section through a spermatosome showing the continued growth of the bundle of axial fibers until it extends beyond the cell.

47 and 48. Sections through two spermatosomes of slightly different ages showing the splitting of the bundle of axial fibers; the sections pass in planes which are at right angles to each other.

PLATE 6.

(Initial magnification 3450 diameters, reduced one-third.)

49. A longitudinal section through a spermatosome in a well-advanced stage. The splitting of the bundle of axial fibers has been completed and the cell is partially filled with the secreted bodies. The nuclear vesicles are rapidly degenerating.

50 to 58. Consecutive cross-sections through a single spermatosome a little younger than that shown in figure 49. The sections are taken in order from the posterior to the anterior end of the spermatosome and show the modifications of the two halves of the bundle of axial fibers as they reach the cell-wall on each side.

59 and 60. Cross-sections through the posterior and anterior halves of two spermatosomes of the same age showing some of the axial fibers moving out beneath the cell-wall.

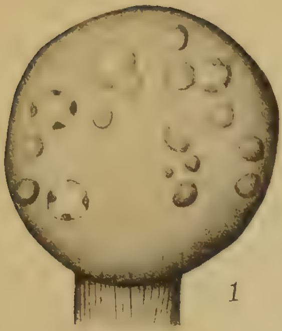
PLATE 7.

(Initial magnification 2450 diameters, reduced one-third.)

63. A longitudinal section through a spermatosome in which the halves of the bundle of axial fibers have reached the cell-wall on both sides throughout their entire length. The secretion of the albuminous bodies has not yet been completed nor has all the chromatin disappeared.

64. A longitudinal section through a nearly adult apyrene spermatozoon showing the total disappearance of the chromatin and the cell filled with the secreted bodies.

65. A longitudinal section through an apyrene spermatozoon of the same age as that shown in figure 64 giving a surface view of one of the two undulating membranes and the longitudinal fibers beneath the cell-wall.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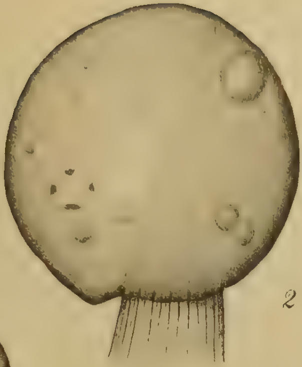
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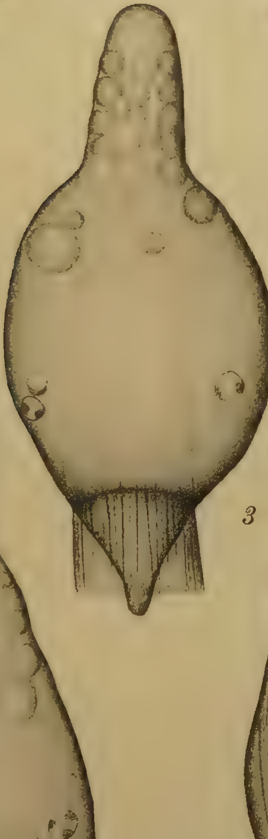
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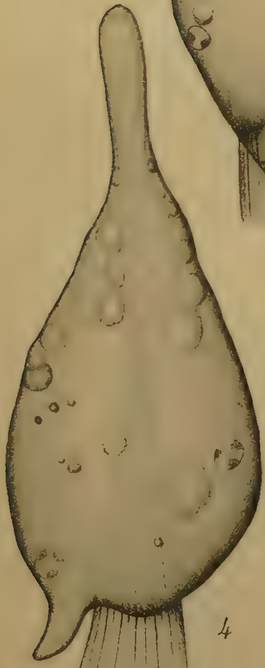
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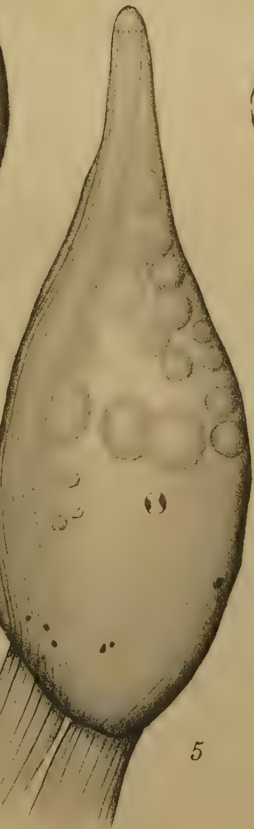
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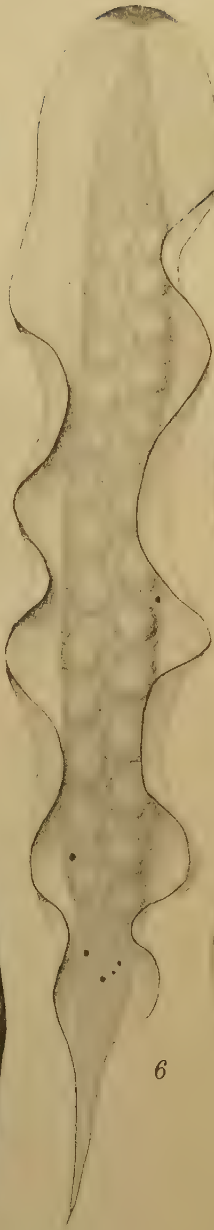
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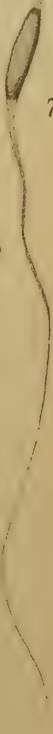
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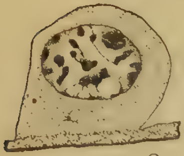
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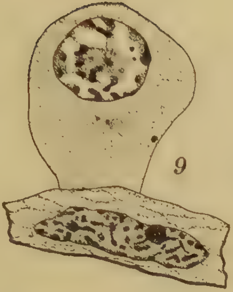
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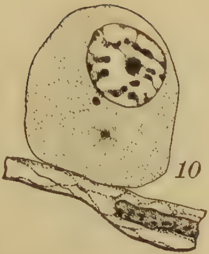
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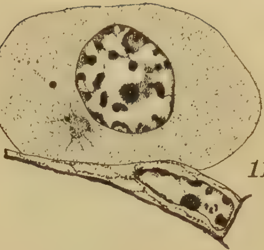
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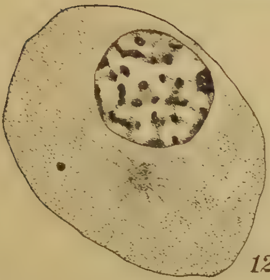
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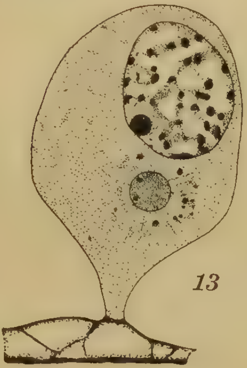
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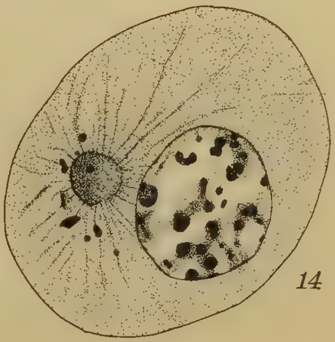
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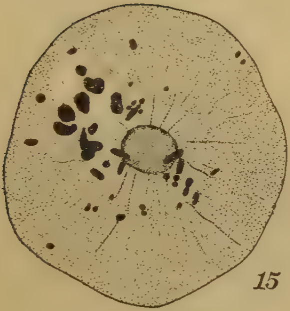
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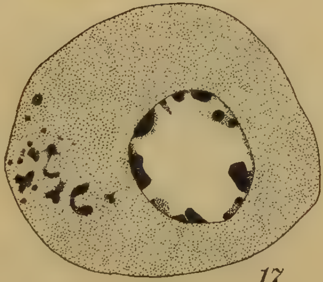
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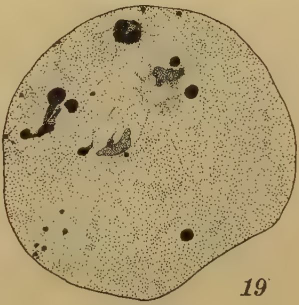
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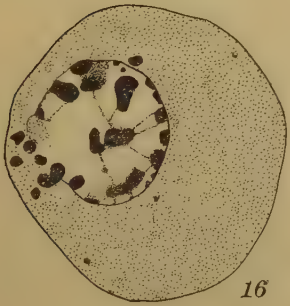
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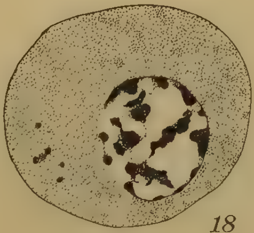
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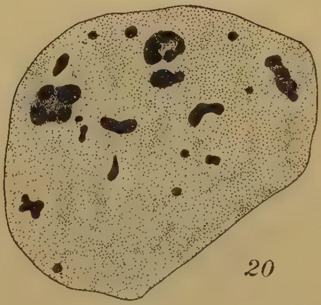
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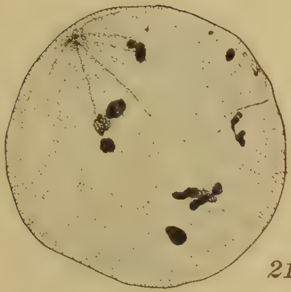
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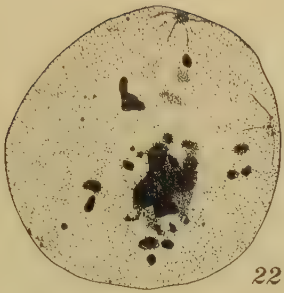
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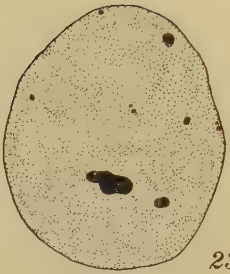
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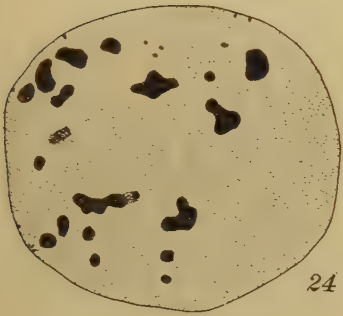
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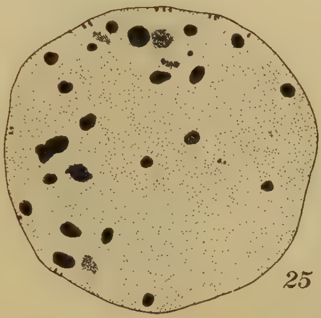
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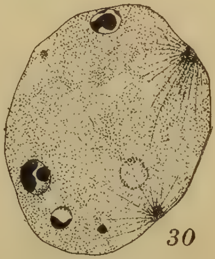
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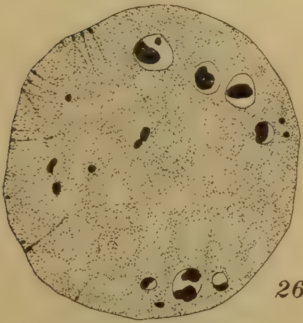
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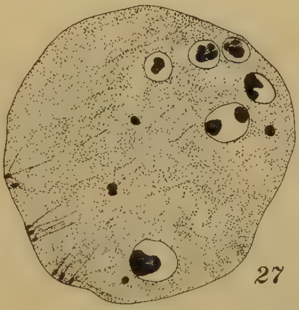
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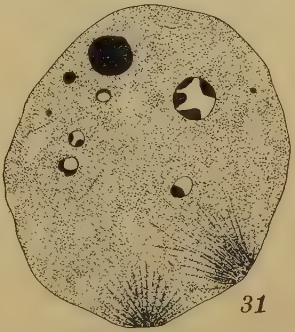
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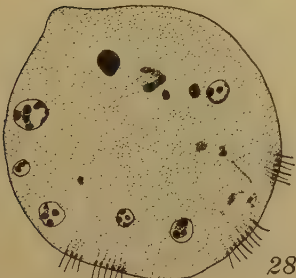
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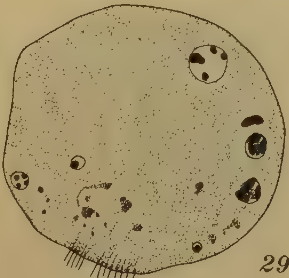
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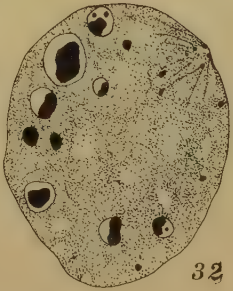
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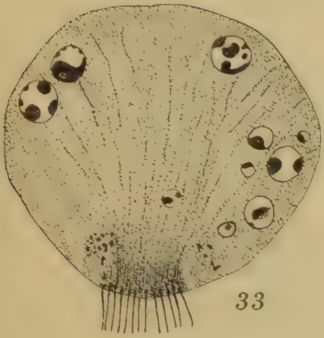
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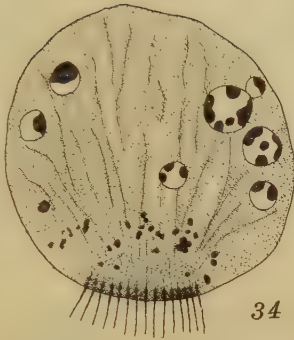
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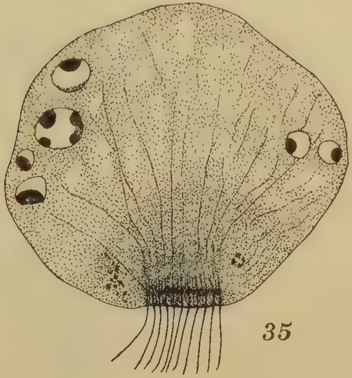
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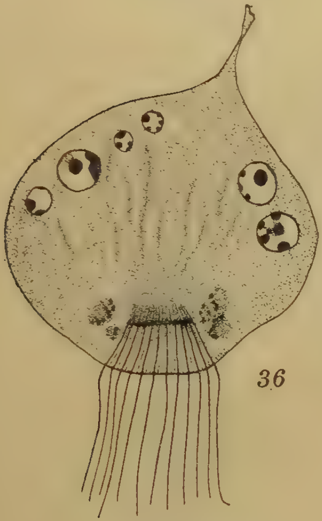
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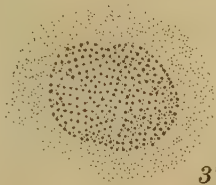
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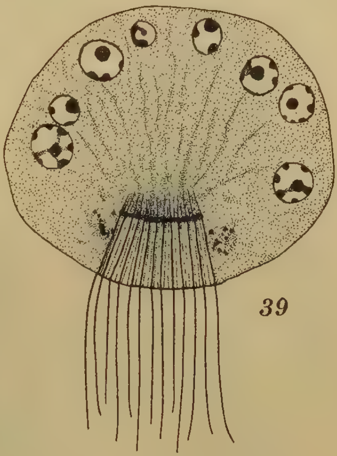
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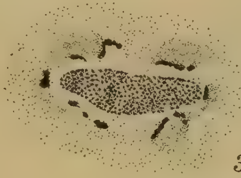
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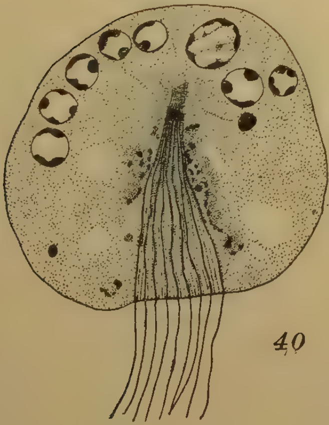
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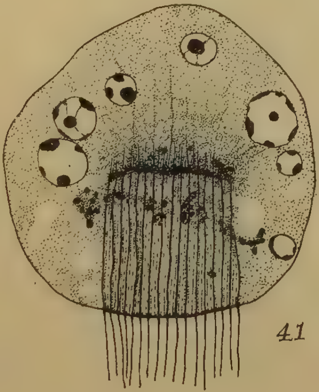
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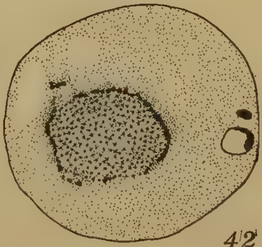
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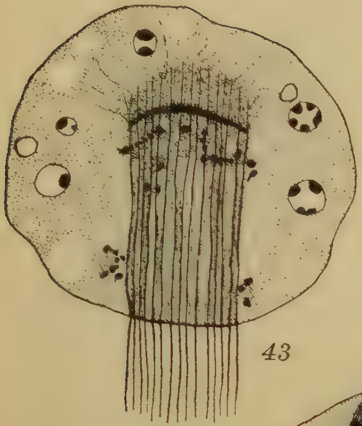

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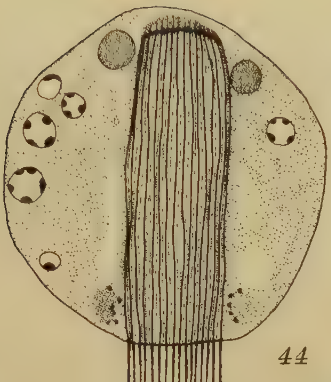

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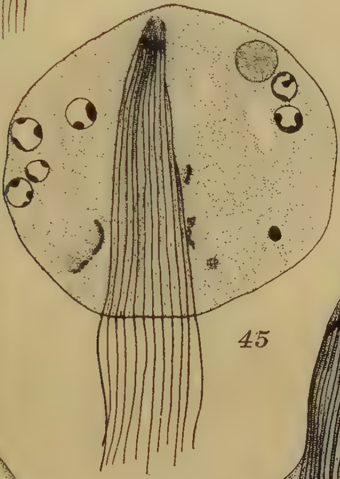

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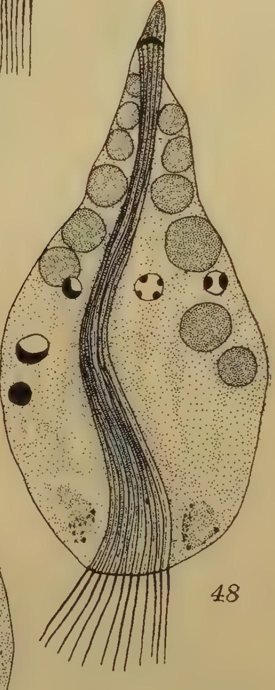

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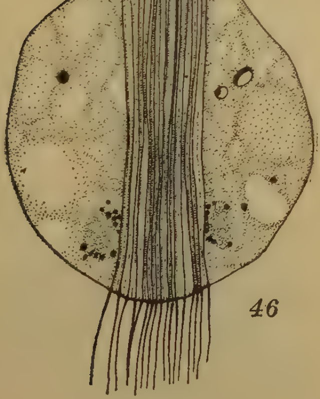

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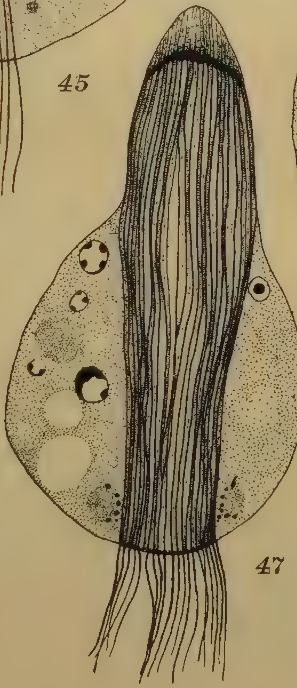

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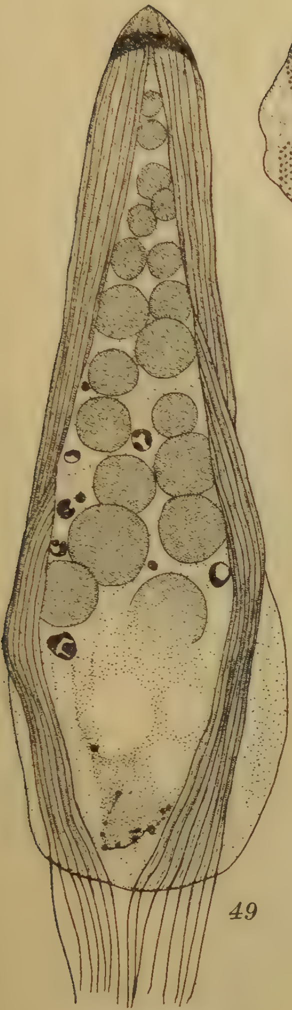

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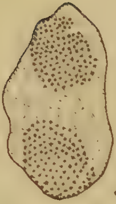

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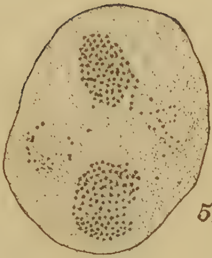

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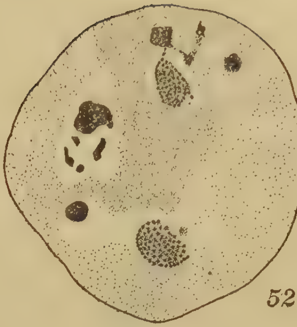

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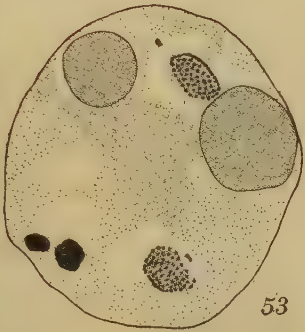

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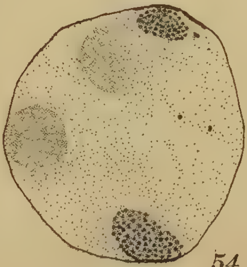

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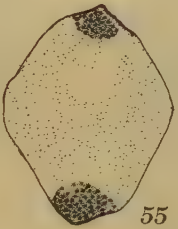

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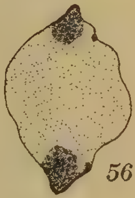

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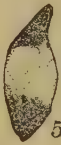

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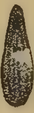

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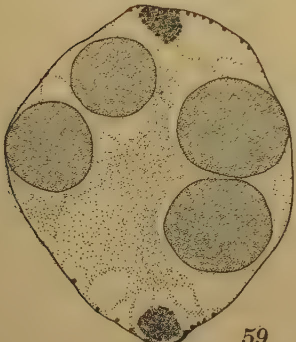

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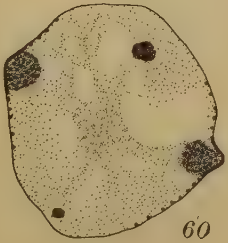

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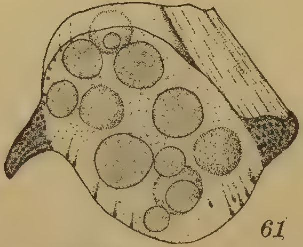

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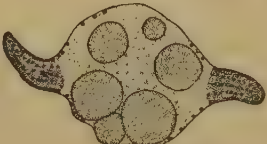

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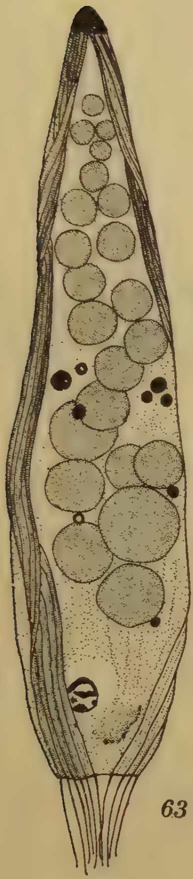

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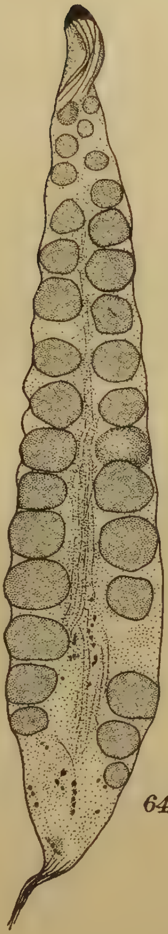

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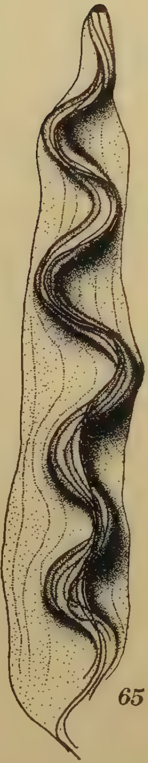

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XII.

HISTORY OF THE SPOTTED EAGLE RAY, AËTOBATUS NARINARI, TOGETHER WITH A STUDY OF ITS EXTERNAL STRUCTURES.

BY E. W. GUDGER,
State Normal College, Greensboro, North Carolina.

Ten plates and nineteen text-figures.

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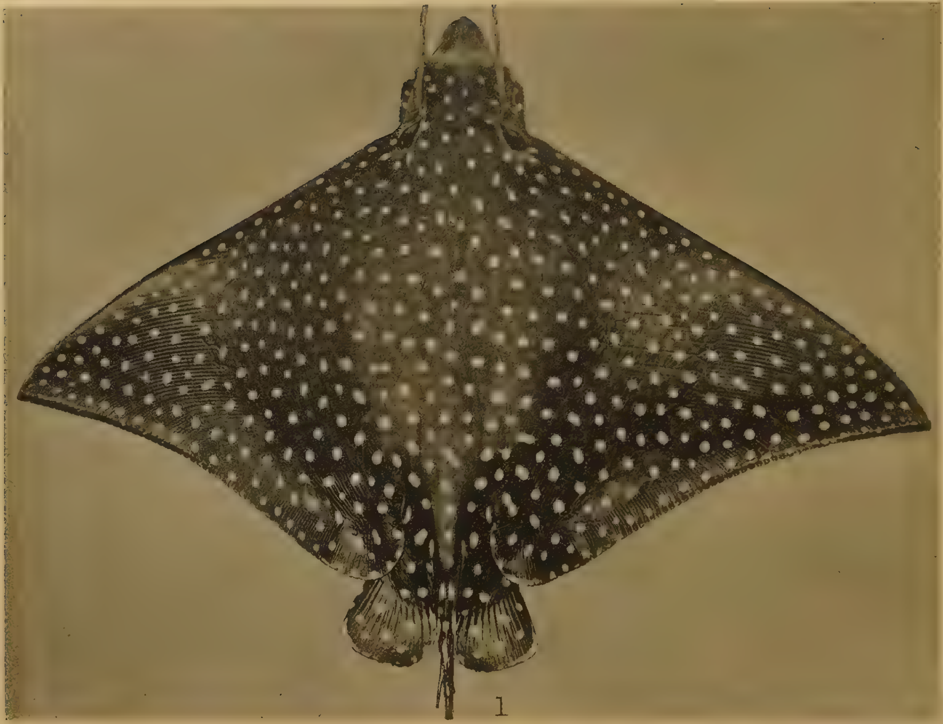

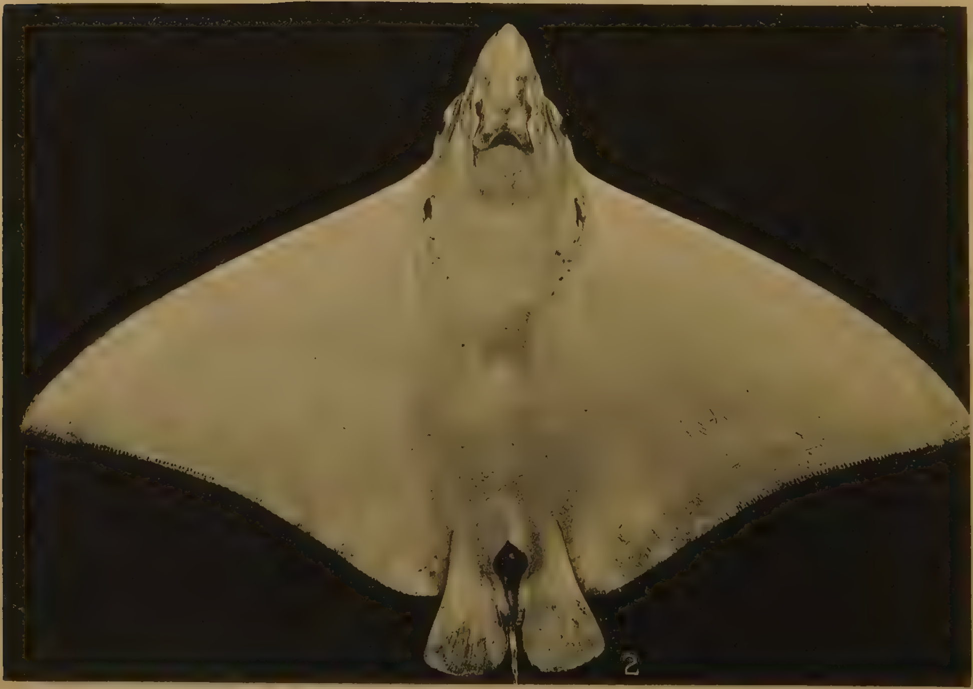

FIG. 1. Dorsal view of *Aetobatus narinari*, from Beaufort.
FIG. 2. Ventral view of *Aetobatus narinari*, from Beaufort.

HISTORY OF THE SPOTTED EAGLE RAY, AËTOBATUS NARINARI, TOGETHER WITH A STUDY OF ITS EXTERNAL STRUCTURES.

By E. W. Gudger.

This paper had its inception in the desire to record permanently certain observations which I was so fortunate as to make on this ray at the Laboratory of the United States Bureau of Fisheries, at Beaufort, North Carolina, in the summer of 1909; but, it was very quickly seen that the value of the paper would be greatly increased if it included a review of the better-known literature of the fish. Before this could be done, however, I again found myself at Beaufort (1910), where I was more fortunate than ever in getting specimens. Later (1913), while working at the Marine Laboratory of the Carnegie Institution of Washington at Tortugas, Florida, a number of fine specimens were obtained from Key West, making possible a comparison of Florida and North Carolina forms. Meanwhile the study of the literature proved of very great interest, and finally it was determined to make the paper include a review of all previous work on this ray. To this end neither time nor effort has been spared and it is believed that the paper justifies its title, since in it every scientific article has been reviewed and every figure of the ray reproduced (with three exceptions noted later), which so far as I know have ever been published.

In such a work, done under many difficulties and at great distances from libraries, I am under many obligations to many people, and it is a pleasure to acknowledge my indebtedness to those who have made this paper possible. To Dr. Theodore N. Gill and Dr. Barton W. Evermann I am indebted for much invaluable advice and suggestions. To the United States Bureau of Fisheries I wish to express my thanks for the fact that the facilities of its laboratory at Beaufort, North Carolina, and of its library at Washington have been freely put at my disposal. My hearty thanks are due Dr. A. G. Mayer, who not only afforded me every facility of his laboratory at Tortugas and Key West, but whose interest led him personally to procure for me three of my four Florida specimens.

THE SPOTTED EAGLE RAY, AËTOBATUS NARINARI.

Among the selachians, or fishes with exposed strap-shaped gill-slits, the order Batoidei, skates and rays, is easily the most interesting because of the very extraordinary and unusual forms assumed by these flat-bodied, disk-shaped animals. All the families of North American rays are represented along the shores of eastern North Carolina, but of them the eagle rays, myliobatids, are the most attractive. These are readily recognized by their wing-like pointed pectoral fins, their long, slender, whip-like tails armed with one or more serrated spines, and their jaws filled with large pavement teeth. Of the three North Carolina genera of the family Myliobatidæ, two forms, *Aëtobatus narinari*, the spotted eagle ray, and *Rhinoptera bonasus*, the cow-nosed ray, have been studied by the writer, while with the third and rarer form, *Myliobatis freminvillei*, he is but slightly acquainted.

Because of its shape and markings, the spotted eagle ray is easily the handsomest of these forms, and, in the whole ray order, so far as represented on the North Carolina coast, its only rival in appearance is the butterfly ray, *Pteroplatea maclura*.

GENERAL DESCRIPTIONS.

The earliest reference to *Narinari*, which the writer has found, is in an old book by Claude d'Abbeville, "Histoire de la Mission des Pères Capuchins en l'Isle de Maragnan,"[1] published at Paris in 1614. On page 245, this old monk writes as follows:

> There is here the *Narinary*, which is another flat-fish very similar also to the Raye. It is six feet long and as many wide, and has a tail about a fathom long, in the middle of which there is, moreover, a spine [as in the preceding ray], but about a full foot in length and very dangerous. This ray is all striped with black and white.

This is very indefinite, since none of the distinctive characters of the fish are given, but the reference is probably to the ray under study. Sloane (1797), from whom the reference came, thinks so. The name, *Narinary* or *Narinari*, is Brazilian, and seems to have been the common designation of this fish. The description of the lines is very interesting, since only one of the several published figures of the fish, that by Jordan and Evermann (1900), shows these lines (text-fig. 3), and but one author, Smith (1907), describes them.

The next reference is found in Jan de Laet's "Novus orbis, seu descriptiones Indiæ Occidentalis," published at Leyden in 1633. In book XVI, chapter 14, page 616, de Laet quotes from Abbeville that *Narrinnari* (also spelled in the margin *Narinnary*) is a species of ray having a tail shorter, but with spines longer (than certain forms previously referred to), and having the whole body marked with black and white lines. Beyond this brief statement he gives no further description, and it is pretty certain that he never saw the fish.

In Purchas (1625), "His Pilgrims," vol. VII, chapter I, is a "Treatise on Brasil, written by a Portugal which had long lived there." On p. 1313 is an obscure reference to a fish which "hath in its mouth two stones as broad as the hand, exceedingly strong, with which they break the Wilkes whereon they feed." And in the next paragraph "these Rayes some have in their mouths two bones, and break with them the Wilkes." These statements

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[1] The island of Maragnan, or Maranham, as the Portuguese wrote it, is the present Maranhao on the northern coast of Brazil.

Sloane (1725), who first notes them, thinks refer to *Narinari*, and it is certain that they refer to the jaw structures of a Myliobatid, or mill-toothed ray.

In 1638, in the capacity of astronomer and naturalist, George Marcgrave accompanied the Dutch expedition to Brazil under the leadership of Maurice of Nassau, who was governor-general of the Dutch conquests in that country from 1637 to 1644. Marcgrave remained in Brazil and northeastern South America for several years. With him went William Piso, who was physician to Count Maurice. While these two men accompanied the expedition in their professional capacities as physician and astronomer, it is certain that as naturalists they had the active aid and cooperation of Count Maurice, as will be indicated later.

Piso's scientific work on this expedition was done wholly from a medical standpoint and is embraced in four books, comprising 132 pages with 104 figures, and bearing the title "De Medicina Brasiliensi." Marcgrave's natural history work is embodied in eight books covering 303 pages, illustrated by 429 figures, and entitled "Historiæ Rerum Naturalium Brasiliæ." Books I, II, and III treat of plants, book IV of fishes, V of birds, VI of quadrupeds and serpents, VII of insects, VIII of the land and its inhabitants, and finally there is an appendix on the Tapuyis and Chilians.

The joint work of Marcgrave and Piso, edited by de Laet, was published in folio at Leyden and Amsterdam in 1648, under the title "Historia Naturalis Brasiliæ." Although Marcgrave's part of this work is more than twice as great as Piso's, he being dead, Piso's contribution fills the first part of the volume and on the title page Piso's name precedes that of Marcgrave.

That part of Marcgrave's work dealing with the fishes of Brazil is the first study ever made of the fish fauna of a region outside of the Mediterranean Sea. He describes 105 species and gives spirited figures of 86 of them, all new to science. To book IV of Marcgrave's "Natural History of Brazil," the student of American ichthyology must go back for the original descriptions and figures of a large number of our fishes. So the present writer has done for the spotted sting ray and the toadfish studied by him. This "Natural History of Brazil," at the time of its publication, was by far the most scientific and comprehensive work of its kind given to the world and it was more than a hundred years before a work of equal importance appeared. Too much praise can not be given Marcgrave and his patron and friend, Prince Maurice, for what even in our day would be a great undertaking.

The Royal Library of Berlin contains two collections of paintings, in folio, of the natural history objects of Brazil. These paintings number 1,460 in all. The larger collection is in oils and bears title as follows "Theatri rerum naturalium Brasiliæ. (Icones in 4 Bänden), Libri Picturati A 32–35." The smaller collection is in water colors and is entitled "Brasilianische Naturgegenstände (Collectio rerum naturalium Brasiliæ in 2 Bänden). Libri picturati A 36–37." In the present writer's biographical sketch of Marcgrave (Gudger, 1912) it is clearly proved that the water-color figures, from which Marcgrave's "Natural History of Brazil" was illustrated, were painted by Marcgrave himself. It seems equally clear that the oil paintings were by a Dutch painter named Franz Poste, who accompanied Count Maurice to Brazil. The whole matter of the paintings is discussed in full in the sketch cited above. These paintings will be referred to later.

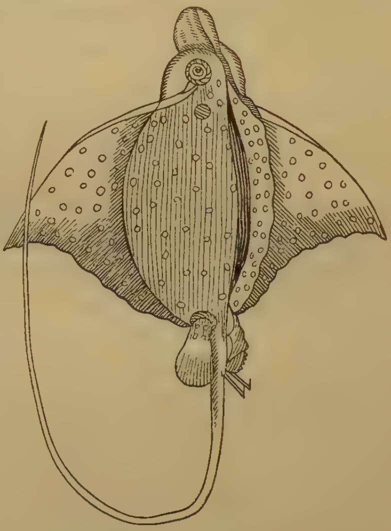
Some time during the years 1638–44 Marcgrave discovered, figured, and described from Brazilian waters the spotted eagle ray, and inasmuch as his original description and figure possess great historical as well as scientific value, since they show what excellent work was done in a wild and barbarous country nearly 275 years ago, by a man working under great disadvantages but possessed of the true scientific spirit, they are herewith reproduced from pages 175 and 176 of the above-mentioned work by Piso and Marcgrave (1648) (text-fig. 1):

Of the several species of fish called "Narinari" by the Brazilians, the one which we have described here is "*Narinari pinima.*" It is called "*Raja*" by the Portuguese, and "*Pylsteerte*" or "*Siecle*" by the Dutch. It is a "*Marina pastinaca.*"

Its body is large, broad, almost triangular in shape, extending out on both sides into very broad triangular wings, which are fleshy in their make-up. Near the tail it has two fins about the size of one's hand, rounded in outline and of equal length. Its head, which is thick, compressed, and furrowed in the middle, is about as large as that of a good-sized pig.

The mouth rounded underneath is triangular, compressed a little, and terminates in a snout. The opening of the mouth is on the ventral surface, 5 inches from the end of the snout. The mouth is 2½ inches wide, toothless, but having in the place of teeth a lower jaw in the shape of a tongue. This is 4 inches long, 1½ inches wide, and reaches to the external opening of the mouth. Likewise, there is an upper jaw placed crosswise, 2 inches long and as many wide.

The lower jaw consists of 17 hard white bones having the shape of the letter U and firmly joined to the membranes. Underneath there lie 17 other bones, one under each, of spongy appearance but not so hard. The upper jaw consists of 14 bones, shaped like the letter J and also joined together by membranes. Likewise 14 other bones lie above these. Moreover the two jaws are joined to the other bones of the head by membranes [cartilages?].


TEXT-FIG. 1.—*Narinari*, from Marcgrave.

The cavity of the skull, wherein the brain lies, is about 6 inches long and hardly 2 wide. The snout is wholly cartilaginous. The fish has two small eyes about the size of a *nummus misnicus.* Behind these eyes, on each side, is a large breathing-hole capable of holding an apple of ordinary size. Within these holes the leaves of the gills lie hidden. On the lower side at the [hinder] end of the head are five oblong incisions.

The whole upper surface of the body is of a steel (*ferreus*) color with white spots the size of a *nummus misnicus* scattered over it, while the under part is entirely white. The skin is everywhere smooth and without scales.

The length of the body from the end of the snout to the root of the tail is 1½ feet; the width between the extremities of the triangular wings is 3 feet 10 inches. The length of the fins near the tail is 7 inches, the width 4. The length of the head is 10 inches, the width 7, and it is 1½ feet thick. The tail is 4 feet 3 inches long and its thickness at the beginning is 5 inches, but it gradually becomes thinner. A little behind the beginning of the tail, there is a small, short fin, a little more than an inch long; and just behind this, standing erect, are two little hooks, curved like fish-hooks and 3 inches long. Its flesh has a good flavor and is sufficient to feed 40 men.

This description needs little or no comment here. It is excellent and would enable one to identify the fish to-day. The statement that the head of the ray is 1½ feet thick, is, inasmuch as the length of the animal is only 18 inches, evidently an error (either editorial or typographical, since the work was published four years after Marcgrave's death). So, likewise, is the statement that the tail is 5 inches thick at the root.

Text-figure 1 is Marcgrave's wood-cut made from the oil painting in the collection referred to. The figure in Marcgrave's text has been colored by hand in one copy of the work found in the Library of Congress, but in the other copy, as in that owned by the Bureau of Fisheries and in the copy belonging to the present writer, the figures are uncolored. The upper surface in the colored figure is a dark steel-blue (*ferreus* Marcgrave calls it) dotted with white or bluish-white spots. Attention should here be called to the discrepancy between the description and the figure in the matter of spines. The former has them curved like fish-hooks, but the figure has them straight, with one large barb on each. As a matter of fact, they are of the ordinary ray type, straight, flat, and multibarbed on the edges.

Figure 3, plate II, is a photograph of the water-color painting to which reference has been made. When it is recalled that this painting was made some 275 years ago, on the wild and inhospitable coast of Brazil, one hardly knows whether to marvel most at the accuracy of the work or the excellency of its preservation. If this figure be compared with text-figure 1, it will be seen how poorly the wood engraver has copied the original painting. Concerning these figures, see Gudger (1912, pages 265–272).[1] The marginal notes on the original painting are presumably those of Count Johann Moritz. Figure 4, plate II, is a photograph of the oil painting in the larger collection described above. The deficiencies of this latter figure, when compared with the former or with my photograph (fig. 1, plate I), are so marked as to call for no comment here.

The older writers seem to have contented themselves with merely quoting Marcgrave and copying his figures. So did Piso himself, in his folio volume entitled "De Indiæ Utriusque re Naturali et Medica," etc., Amsterdam, 1658, though he abridged Marcgrave's original description somewhat. However, he does one good deed in giving us a clue to the meaning of the Brazilian word *Narinari*. He tells us that the tail is armed with two stings "approaching in form the figure of an arrowhead, and therefore these fishes are properly called by us *Pylstaert*, and by the Brazilians *Narinari*." Now since the Dutch word *Pylstaert* means both a water bird and a sting ray, we might think that the allusion was either to the wing-like pectorals or to the spined tail, had not Piso made it clear that the latter is meant. It seems then that the word *Narinari* means sting ray (see Martius, 1867).

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[1] For these photographs and for much information about these drawings, I am indebted to the courtesy of Dr. Perlbach, of the Royal Library of Berlin.

Willughby, in his "De Historia Piscium," published after his death by Ray at Oxford in 1686, reproduces both figure and text with great exactness. The fish, however, is represented as if it were lying on its left and were drawn from the right side. The description and figure are credited to Marcgrave.

The next reference to this interesting fish is to be found in Jonston's "Historiæ Naturalis de Piscibus et Cetis." This work passed through several editions. The first, according to Walbaum, was published at Frankfort in 1649. Three editions are dated at Amsterdam in 1657, 1660, and 1718. In the Latin version in the Library of Congress, bearing date of 1767, Jonston reproduces Marcgrave's figure, but reverses right and left sides, as does Willughby, and takes out some of the crudities of the drawing, and quotes Marcgrave's description almost verbatim, giving him credit.

Henry Ruysch, in 1718, published his "Theatrum Universale Omnium Animalium, Piscium," etc., which seems to be a compilation of the works of various writers from Aristotle to Marcgrave and Jonston, but mainly of the latter. Ruysch has rearranged Marcgrave's description and figures, probably following Jonston therein. He has copied Marcgrave's description of *Narinari* in the minutest detail, but without a word of reference or acknowledgment. He reproduces Marcgrave's figure, but has improved it, especially in the shading.

In 1697 Hans Sloane figured and described in volume XIX of the "Philosophical Transactions" the jaws of a *Pastinaca marina* from Jamaica, which he says is identical with Marcgrave's *Narinari*. In his brief description of the fish he says that it is "smooth, blue; covered with white spots." Later (1725), in volume II of his Natural History of Jamaica, he describes this fish more fully.

> This [whip ray] was about two foot over from corner to corner, and all blue even the flesh itself with white spots on it, the under side or Belly was white, as in others of this kind, the tail was six foot long, black, small and smooth, of which are made whips, whence the name Whip-Ray, beyond the Pinna at the end of the body or in the beginning of the tail lie one, two or three inch and a half long flat streight bones or Radij; they are white, serrated with Teeth on both Sides like a saw, made so as an arrow that's barbed, to enter the flesh easily but not to come out without tearing it, they lie one on another on the upper part of the tail, where there is a hollow or cavity made to receive them like a sheath, that they may swim with less Impediment and only use them on Occasion.

It is probable that Patrick Browne, in his "Civil and Natural History of Jamaica" (1756), refers to our ray when, on page 459, he describes a whip-ray thus: "Middle parts bluish mixed, tongue long, with a barbed spine on the finned tail." Ray (1713), who it will be remembered edited Willughby's manuscript, in his "Synopsis Methodica Piscium," quotes Sloane and Marcgrave, but adds nothing new concerning our ray.

I find no other reference to *Aëtobatus narinari* until 1790. Excepting Abbeville, Marcgrave, and Sloane, and possibly Piso, none of the older writers above quoted seems to have ever seen the fish; they were all compilers. However, in the above-named year, a Swede, Bengt Anders Euphrasen, described and figured a specimen of this ray which had been taken at the island of St. Bartholomew, one of the West Indies at that time belonging to Sweden. He calls this fish *Raja narinari*, thus fixing the specific name. He refers to Willughby, whose description and figure he criticizes, seemingly in ignorance of Marcgrave, from whom Willughby copied both figure and description.

Euphrasen's figure, which was drawn in Sweden from a preserved specimen, is herewith reproduced (fig. 5, plate III), but modified slightly in one unessential detail. In the original figure, the long tail hangs down straight save for a fish-hook-like curve at the tip; in the reproduction, for the sake of economy in space, it is curled to the left as shown; the length, however, is correct. Euphrasen describes his specimen as follows:

> Head prominent, compressed, in form like a toad's, on top 2 pits placed transversely. Mouth ventral as in its kind, transverse, teeth few and closely crowded. Eyes lateral, of medium size when compared with the head. Ears (*aures*) above the neck, or at the base of the head, with apertures round, and a little larger than the eyes. Spiracles 5, under the throat on both sides as in its kind.
>
> Body flat, very broad, ending laterally in acute angles, the margin feathered (*pinnis*). Above brown[1] (blue) with round white spots, in diameter about the size of the thumb, scattered about; beneath white. Anal fins one on each side at the base of the long tail, reaching to the base of the spine on the anterior part of the tail. Dorsal fin small, subtriangular, situated on the base of the tail. Spines 2, oblique, dorsally placed behind the fin, compressed, retrorsally barbed, the posterior double the length of the anterior. Tail whiplash-like, compressed, attenuate from base to apex, without fin, 3 times longer than body.

In Walbaum's edition of Artedi's "Bibliotheca Ichthyologica," part III (1792), the former quotes the name *Raja narinari* from Euphrasen's paper above named, refers to Willughby and Marcgrave, and gives this brief description: "Body smooth, steel colored above, with numerous white spots."

About this time, *i. e.*, the close of the seventeenth and the beginning of the eighteenth century, a number of ichthyologists include *narinari* in their lists of rays without adding anything to our knowledge of the fish and some of them fall into the grievous error of applying the name to rays which are not even eagle rays much less Aëtobatids. Bloch in volume III of his "Ichthyologie" (1786) appends the name *narinari* to an ordinary sting ray with a long tail, and which he expressly states has *many* rows of teeth in its jaws. Other cases might be cited, but to no purpose.

In the closing years of his life, Bloch had outlined and made some progress on a great ichthyological synopsis after the plan of Linnaeus's "Systema Naturæ." After his death, this important work was brought to completion by J. G. Schneider (Bloch and Schneider, 1801). Beginning on page 361 we find the description of *Raja narinari*. This I believe to be the work of Schneider for two reasons; first because in Bloch's other works there is nowhere found any description of *A. narinari*, and in the second place because the account under discussion is written in the first person.

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[1] Euphrasen uses the word *chalybeum* from chalybs, meaning steel or iron, but as his figure is a sepia-brown it may be that the color of his fish was brown.

The description of this ray is interesting not because it contains anything new or unusual or erroneous, but because it is a curious jumble of Marcgrave, Willughby, Euphrasen, J. R. Foster (MS. "Descriptiones Animalium"), Prince Maurice of Nassau, and Hans Sloane ("History of Jamaica"). However, Schneider examined several "Swedish specimens," and among them probably Euphrasen's, while he also had several sets of jaws and a dried specimen cut in half.

Omitting those parts of Schneider's description, which will be taken up in the special sections, his general account is now given. He says that the head is compressed and prominent; above the apex there may be noted holes (spiracles) placed transversely; the mouth is inferior and is filled with a few teeth crowded together; the laterally placed eyes are of medium size in round orbits but little larger than themselves; the body is broad and flat, the lateral angles are acute, the emarginate fins are steel-colored above with round white spots the size of the thumb, and the tail is three times longer than the body. Its habitat is given as the American Ocean, especially around the Caribbean Islands.

In 1803, the East India Company published in London, in two sumptuous volumes, "Descriptions and Figures of Two Hundred Fishes Collected at Vizagatapam on the Coast of Coromandel" by Patrick Russell, a physician in its employ. On page 5, there is described a ray called by the natives *eel tenkee* and by Russell the *ocellated raja*. Figure 6, plate III, is a reproduction of plate VIII of the above work, showing this fish. That this is *Aëtobatus narinari*, inspection of the figure and study of the description make clear. The general outline of the fish, the pointed pectorals with fimbriated hinder edges, the white spots profusely covering the body back of the shoulder region, the projecting head with its pointed snout, the relative position of the eyes and spiracles, the rounded ventrals with the dorsal placed well between them on the root of the tail, all spell *narinari*. When one reads that the tail is long and whip-like, and that the jaws are dissimilar, "the lower arched, narrow, and projecting beyond the wider immovable upper jaw," one is quite sure that it is *narinari* despite the absence of spots on the anterior dorsal region and the fact that both jaws are described as devoid of teeth. These points will be considered later.

Blainville, in the Journal de Physique, tome 83, for 1816, has a systematic paper in which he establishes the genus *Aëtobatus*, assigns the generic characters, and notes ten species, of which *narinari* is one. Later, Blainville (1828) changed the generic name to *Aëtobatis*, though a careful search through his paper fails to reveal any reason therefor. His characters for the genus are as follows:

> Body together with the pectoral fins in the shape of a bird with extended wings; head free and provided with a simple appendage in front; eyes lateral; teeth large, smooth, polygonal, united into two plates, one lingual, the other palatine; pectoral fins pointed, anterior border convex, posterior concave; pelvic fins as in the sting rays, only one fin above on the root of a tail often very long, flagellate, armed with one or two spines.

This, it should be noted, is a statement of the characters of the genus only; *narinari* is not named and no specific description is given save for *Raja aquila* of European waters.

In the celebrated voyage around the world of the corvette *Uranie*, a spotted sting ray was taken at Guam. The zoology of this voyage was written up by Quoy and Gaimard (1824), the ship's surgeons. They described their ray as having an elongated snout, while its general color was a light brown sprinkled with round spots of cerulean blue, and its tail was armed with five very long, barbed spines. Their description is very imperfect and their drawing "made on the spot" was lost. They were much impressed by the fact that this ray had five spines on the tail and refer to it as such an extraordinary matter as to make the tail worthy of deposit in the Museum (of Paris?), and from this phenomenon gave to the ray the name *Raja quinqueaculata*, the 5-spined ray. A photographic reproduction of their fine figure of the spines is given in figure 7, plate IV, of this paper. Below in the same figure is a photograph of a portion of the tail with four stings of *A. narinari* from Beaufort. In the section dealing with the tail and its spines, a careful comparison of the two tails will be made.

In 1835, Ruppell described an eagle ray from the Red Sea under the name *Myliobatis eeltenkee*. However, he has doubts about the identity, for he says that he is not able to say whether it is different from Linne's "form called *Raja narinari* after Marcgrave's description."[1] In his synonymy, Ruppell says his fish is identical with Russell's *eel tenkee* which Gunther says, and which we have just seen, is *Aëtobatis narinari*. That it is *A. narinari* can not be doubted when one reads of the long, pointed pectorals with fine notchings on the posterior edges (found on the ventrals also) the white spots covering the dorsal surface; the elevated head with lateral eyes; the long, pointed snout, and the mouth with its teeth in a single row in each jaw, the lower being angled outwardly. Some doubtful points are seven teeth only in each jaw (these will be considered at length later), the dark olive color above, and the flabelliform tail.

Despite the fact that Swainson (1838–1839) spent some months on the northern coast of Brazil in 1816–1817, there is no evidence that he ever saw our ray. He quotes Russell, and also Müller and Henle, whose great work was then appearing in parts, but adds nothing to our knowledge.

In 1841 the celebrated Johannes Müller, with the cooperation of Jacob Henle, published at Berlin in folio their "Systematische Beschreibung der Plagiostomen." In it they definitely established the form *Aëtobatis narinari* based upon the examination of twelve specimens from Brazil, the Indies, and the Red Sea, six being in alcohol and six dry. The congeneric *Raja flagellum* of Bloch and Schneider, which it must be remembered is declared to be identical with *A. narinari* by Günther and by Jordan and Evermann, is based on the examination of 19 specimens and one head.

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[1] In none of the several editions of Linne's "Systema Naturæ," which I have examined, is there any record of *narinari*.

They describe the upper surfaces of their specimens of *A. narinari* as brown with round, white, regularly scattered spots which are lacking only on the head, and add that the number of the spots is often small. Both my photographs and Duméril's figure (reproduced as fig. 8, plate IV, of this paper and referred to elsewhere), show that the head does not lack for spots and that the number on the body is great. Curiously enough, however, Jordan and Evermann's figure (text-fig. 3) shows very few spots in the cephalic region. The fish from which it was drawn, however, like the twelve examined by Müller and Henle, was a preserved specimen.

In the years 1772–1774, Johann Rheinold Forster collected fishes in the southern seas and later described his collection. His manuscript, however, remained unpublished, though noted by Bloch and Schneider (1801), until it was edited by Lichtenstein and published at Berlin in 1844. He collected both in Brazilian waters and in the South Pacific, and described and figured a fish which he called *Raja edentula*, but which is easily recognizable as *Aëtobatus narinari*. According to his description, this ray is lead or steel colored above with many round white spots, white below, and everywhere smooth and bare; the head, slenderer than the body, terminates in a triangular blunt snout, cartilaginous in structure, and having the edges rolled up. The mouth is typically that of *narinari;* the crescent-shaped lower teeth form a spatula projecting beyond the shorter and broader upper jaw. The eyes are lateral, prominent, and rather far back and have vertical pupils.[1] Behind the eyes are the spiracles, connected with each other and with the mouth. The tail is three times as long as the body, slender, cylindrical, pinnate (dorsal?), black, and armed with two serrate spines. Pectoral and ventral fins are crenate behind. This is an admirable description of *A. narinari*, but unfortunately accompanied by no figure. Lichtenstein does not state why the figures, made to accompany the text, were not published.

Cantor, in his "Catalogue of Malayan Fishes" (1849), describes an East Indian Ray, *Stoasodon narinari*, which seems to be identical with our ray. His description need not detain us here longer than to note that his fish is greenish-olive or greenish-gray above, while the greenish-white spots edged with black are found on all parts of the dorsum save the head and anterior margin of the pectorals. These points will be taken up later.

Bleeker (1852) declares that *A. narinari* is common throughout the East Indies. His description of this fish does not differ from that given by other authors save as to the color of the body and the structure of the jaws, consideration of which points will be entered into later. Although he gives no figure of his fish it is undoubtedly *A. narinari*.

The most elegant figure of an eagle ray that has ever been published is that of August Duméril found in tome 10 of the "Archives du Museum d'Histoire Naturelle" for 1858–61. Figure 8, plate IV, is a photographic reproduction of plate 20 from the above volume. *A* is *A. latirostris* from the region of the Gaboon (river), *B* (lower left corner) is a head of *A. nari-*

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[1] This is the earliest note made of this peculiarity.

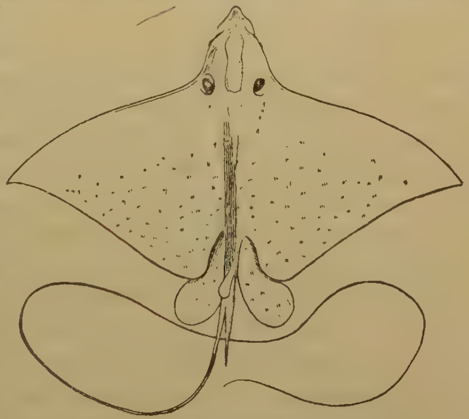
nari, *C* (lower right corner) is *A. fouet*, (*A. flagellum*?). One observes at once the larger spots of *A. latirostris*, and the fact that they are few in the cephalic region and rather widely scattered over the other parts of the body, while in *A. narinari* they are much smaller, thickly scattered, so much so on the head as to appear concentrated. Worthy of note is the fact that the congeneric form *A. fouet* is entirely devoid of spots. These differences will be discussed further in the section dealing with the species.

In 1865, Day in his "Fishes of Malabar" and Duméril in his "Histoire Naturelle des Poissons" both described *Aëtobatis narinari*, but as the distinctive points in their description have to do with the color and with the structure of the jaws, both of which subjects will be discussed fully later, and as neither published plates, further attention will not be given them here.

In the eighth volume of his "Catalogue of Fishes in the British Museum," Günther (1870) gives a description of *Aëtobatis narinari*, which, since it is based on a careful examination of no less than ten specimens (mainly from oriental waters), is possibly the most authoritative we have had since Müller and Henle. In general he agrees with the writers cited above, but gives some interesting variations in color and teeth which will be considered at some length later.

Klunzinger (1871) gives a very careful and exact description of *Aëtobatis narinari* from the Red Sea. This does not differ materially from Ruppell's earlier account (1835), but goes into much greater detail. It is interesting to note that both writers record the fact that the spiracle is broader than long; Ruppell says that it is pear-shaped. The points of chief interest in Klunzinger's description have to do with teeth, tail and spine, and color, all of which will be dealt with later in their appropriate sections.

Day (1878), in his "Fishes of India," gives a description of *Aëtobatis narinari* based evidently upon a number of specimens from Indian waters. These, however, were probably preserved specimens, since he speaks of the tail as triangular as far as the spine and compressed beyond that, structures much plainer in preserved than in fresh specimens. His figure was certainly made from a preserved fish, since it is much shrunken in the head region. The spine is disproportionally large and the dorsum is entirely devoid of spots despite the fact that in his description the body is noted as covered behind the head with dirty white or bluish spots. This figure is hardly to be recognized as *A. narinari* and it has not seemed necessary to reproduce it here.



Text-fig. 2.—*A. narinari*, after Gunther, 1880.

In 1880, Gunther published his "Introduction to the Study of Fishes," but therein adds little to our knowledge of this ray. His figure (text-fig. 2) is a mere outline drawing, but is one of the best for general appearance which we have. Judged by my specimens, the ventrals are too long and too rounded, the spine too large, the depression over the brain too accentuated, while the absence of spots from the anterior part of the body is especially noticeable. The snout is good and the eyes, it should be noted, are almost invisible, as in the living fish. The same figure is given in this author's (1886) article on Rays in the ninth edition of the Encyclopædia Britannica.

After giving the general characters of the family *Myliobatidæ* and of the genus *Aëtobatus*, Jordan and Evermann, in their great work "The Fishes of North and Middle America" (1896), thus describe *Aëtobatus narinari:*

> Disk twice as broad as long, its anterior borders a little convex, posterior concave, outer angles pointed. Cephalic fin about one-third broader than long. Teeth of lower jaw straight or more or less angularly bent. Tail three or four times length of disk. Brown with small round pale spots (Duméril). Tropical seas, north on the Atlantic coast to Virginia: not very common on our shores. Narinari, a Brazilian name.

Their figures, both dorsal and ventral, are herein reproduced as text-figures 3 and 4. They were made from a preserved (alcoholic?) specimen taken near Cedar Keys, Florida, and deposited in the United States National Museum. Beyond calling the reader's attention to the parallel dark markings on the dorsum, which are not mentioned in the text, criticism is deferred until later.

The writer has elsewhere (Gudger, 1910) published measurements and brief descriptions of two spotted sting rays taken at Beaufort in 1909, and of another taken at an earlier date. The largest of the three (sex unknown) was taken by some fishermen in the deeper part of the outer harbor in September 1901. Its body was 2 feet 2 inches long and 4 feet wide, while the tail was 4 feet 8 inches long. The second, a female, was taken by the writer in a seine on June 12, 1909, at the Narrows of Newport River, a wide-mouthed tidal estuary whose lower reaches form part of Beaufort Harbor. When just out of the water, its measurements were as follows: length, snout to the tip of the ventrals, 18 inches; width over pectorals 26 inches; length of tail 40½ inches.

The third specimen, also a female, was, on July 3, 1909, brought to the Fisheries Laboratory by Mr. Russell J. Coles, of Danville, Virginia. A few days previously he had caught it on a hook in the Bight of Cape Lookout, and rightly thinking it to be an unusual form, had preserved it in a tank of formalin loaned him by the laboratory and had brought it in for identification. This fish was 23½ inches wide and 16 inches long, with a tail measuring 35½ inches. A fourth specimen, of the same sex as the preceding, also taken at Cape Lookout (in 1906), is recorded in the card catalogue of fishes at the Laboratory, but no measurements are given.

In 1910, I had the good fortune to get three fine specimens of this fish, at the capture of two of which I was present, while the third was brought to me some 30 minutes after death. These rays have been previously described (Gudger, 1912A), but their measurements will be given here and their markings discussed in the section dealing with color and spots.

No. 1, a female, was taken about half-way between the laboratory and the Narrows of Newport River. It was 28½ inches wide, 19 inches long, with a 33 inch tail. The total length was 49 inches and its weight 11½ pounds. It had two spines.

No. 2 was a male taken in the channel connecting the inner and outer harbors. Its width was 27½ inches, length 18½, tail 39½, total length 54½ inches. It had two spines.

No. 3, also a male, was taken within 200 yards of the hauling ground where No. 2 was seined. Its width was 37 inches; length, snout to ventrals, 26½ inches, tail only 27¾, over all 49¾ inches. The width between its eyes was 5 inches, the longest diameter of the spiracle was 1¾ inches, and its weight was over 25 pounds—the limit of my spring balance. This ray had three spines, the middle one of which was torn loose, as was the anterior spine of No. 2 above. Its tail was very short, far too short in proportion to the other dimensions if the fish be compared to a normal ray. Whether or not this abbreviation was the result of an accident, can not be said, since there was nothing to indicate the cause. Interesting points concerning the color, number, size, arrangement, and position of spots of this ray will be taken up in the section dealing with the color. This specimen was the largest and finest of the five which I have had from Beaufort waters.

Coles (1910), who (as noted above) had furnished me with a fine specimen early in July 1909, had the good fortune to take more than fifty specimens during that season. During July 1911, he saw nearly as many more but only took eight. The largest of these, a female without eggs or embryos, was 5¾ feet wide, 3 feet long, tail 5¾ feet long, total length of body 8¾ feet, weight 132 pounds.

In the summer of 1912, Mr. Coles captured a number of spotted rays at Cape Lookout. The measurements of the three largest are as follows: first specimen, a female, 9½ feet long from tip of snout to end of tail, and 7 feet 2 inches wide; the second, also a female, was 12 feet long over all, and 7 feet 7 inches wide, and 20 inches thick measured on a lance thrust through the body; the third, a male, was 10 feet long, and 6 feet 11 inches wide. It is to be regretted that measurements of the body proper and of tails alone were not made. These giant rays would probably have weighed 400 to 500 pounds each. Further consideration of these specimens will be taken up in the section on markings.

The specimens taken by Coles and myself, together with certain observations of his noted elsewhere (Coles, 1910), indicate that these rays are far more abundant in the Beaufort region than I had previously thought (Gudger 1910 and 1912A). Their relative abundance along our coasts at certain seasons of the year is also testified to by various fishermen of Beaufort and Morehead City.

My Florida specimens agree in general characters with those from North Carolina. The largest (and the largest I have ever seen) measured as follows: width 5 feet 2 inches; length to tip of ventrals 3 feet 8 inches; length of tail only 6 feet 10 inches; length all over, 9 feet 9 inches; width between eyes 8⅞ inches, between spiracles 5½ inches; weight 120 pounds. The others need not detain us here. Their measurements are given in the table on page 261. Details of color and number and size of teeth, etc., will be taken up in the sections dealing with these structures. It may be remarked in passing that these rays seem to be abundant about Key West. Dr. Mayer, in one afternoon in May, off Slaughter-house Point, secured three from fishermen using the grains. At the same place a month later I took the large one above referred to. Dr. Mayer's specimens were preserved in a rather unique way. They were small rays and this made it possible to suspend them in a can at the ice factory in Key West where they were frozen solidly in a 300-pound block of ice to await my coming some weeks later.

Figure 1, plate I, which serves as a frontispiece to this paper is reproduced from a photograph of my specimen No. 3 of 1910. Figure 2, plate I, is a ventral view of specimen No. 2 of 1910, while figure 13, plate VI, is a lateral view of Coles's 1909 specimen. My specimens were photographed while perfectly fresh, in fact within an hour after being taken. The specimen from which the lateral view was made had been in formalin for a few days, but so far as could be told was quite normal in all respects.

The photographs reproduced in figures 1 and 2, plate I, were made as follows; to a post standing just before the largest window in the laboratory a cross-piece was nailed; over this was hung a white sheet to serve as a background; in front of this the fish was suspended from the cross-piece by a cord run through the spiracles and their inter-communicating passage. The ray was first suspended by fish-hooks caught in the upper gill-slits, but as the very considerable weight of the fish caused these to tear the flesh, as shown in the ventral view, they were discarded. The method used in getting the lateral view is clear from the figure. The fin is turned up to show the gill-slits.

If figure 1, plate I, be compared with the various illustrations reproduced in this article, some interesting comparisons may be drawn. In Marcgrave's figure (text-fig. 1), it will be seen that the general outlines are good, even though the drawing is crude. His figure is drawn from a "quartering" view, *i. e.*, from a point above, but about 45 degrees to the left. He says that the whole upper part is of a steel color and that scattered over the whole of this are white spots. The anterior borders of the pectorals are too convex, the posterior edges too concave, the angles too acute. The snout is too blunt, the eyes and spiracles too high on the head. The fore and aft striations were not found in any of my specimens but are shown in Duméril's figure (fig. 8, plate IV). But with all its defects, even to a novice, text-figure 1 is plainly a drawing of an eagle ray; and when the description of the teeth is taken into account, it is unmistakably *Aëtobatus narinari.* But if comparison is made with Marcgrave's original figure, the water-color drawing shown in figure 3, plate II, the excellence of the latter will be greatly emphasized.

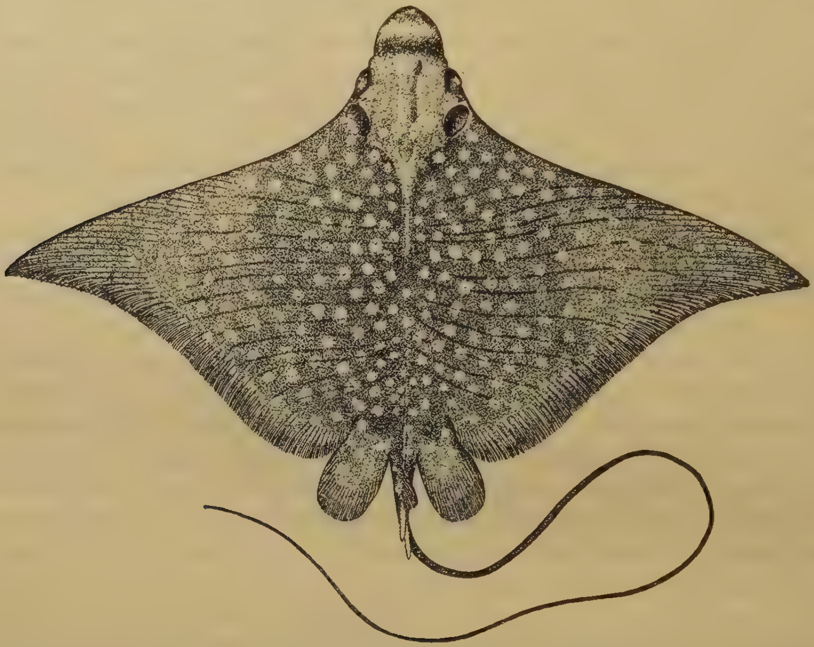
As indicated above, the older writers merely copy Marcgrave, and those who do not do worse, for quite a number of them, notably Bloch, apply the name *narinari* to rays which are not only not Aëtobatids but in some cases not even Myliobatids. It will be remembered that Euphrasen declared his figure of the spotted sting ray to be much superior to Willughby's, which is merely Marcgrave's figure redrawn and somewhat touched up. But if the figure in question (fig. 5, plate III) be compared with my photograph or Duméril's drawing of *Aëtobatus narinari* or Marcgrave's water-color drawing, some extremely grave faults will be revealed.

In figure 5, plate III, body, head, and pectorals seem to be on a level. Both the anterior and posterior edges of the wings are too flat in outline, while the ventral fins are shown as bifurcated backward prolongations of the body. Inspection of figure 1, plate I, shows them to be short and clearly marked off from the body. The dorsal fin is too long and is wrongly inserted. Figure 1, plate I, does not show it clearly, but text-figure 3 (from Jordan and Evermann) shows both size and position properly. Where Euphrasen got the fringed edges of the pectoral and ventral fins is inexplicable. It is, however, probable that his figure was drawn from a much shrunken, dried or preserved specimen in which the fin rays were very prominent; but even that would not account for their extending beyond the substance of the body proper. Inspection of figure 1, plate I, shows that the fins in question possess slightly notched edges but nothing more. Attention is also called to the exaggerated size of the spots, to their relatively small number, and to the fact that there are practically none on the head; also to the position of the spiracles and eyes, which are dorsal instead of lateral, the eyes in the fresh specimen not being visible from above. Further the snout is too short, too narrow, and too much on a level with the main part of the head.

Russell's (1803) Eel Tenkee (fig. 6, plate III) is a good drawing with some rather apparent defects from the standpoint of my specimens. The body is too flat, and the head especially so; the anterior parts lack spots, and the eyes are too prominent; posteriorly the line of demarcation between pectorals and ventrals is continued too far forward. However, the crenate posterior edges of the fins are correctly shown. This drawing was made by a native artist, but Russell especially vouches for the accuracy of the details.

Duméril's elegant figures (fig. 8, plate IV) have already been referred to on page 252. For the reasons noted elsewhere it seems to me that *A*, *Aëtobatus latirostris* is not (as Gunther asserts) *A. narinari*, but that *B* (lower left head) is *narinari.* The drawing was evidently made from a preserved specimen, in which by drying or hardening the flesh had sunk over the anterior fontanelle of the skull. The position of the spiracles is for the same reason somewhat distorted.

Gunther's excellent little outline drawing (text-fig. 2) has been previously referred to and need detain us but a moment. One's attention is forcibly called to the absence of spots on the anterior parts, to the elongated and very *rounded* ventrals, and to the exaggerated spine. The dorsal fin and head are well drawn, the eyes being almost invisible, but the spiracles are placed rather too near the mid-dorsal line.



TEXT-FIG. 3.—*A. narinari*, dorsal view, after Jordan and Evermann (1900), Florida.

The only other figures of this fish (and the only drawing of the ventral surface known prior to 1913) are those previously referred to from Jordan and Evermann's "Fishes of North and Middle America," and widely reproduced in various papers by one or the other of these ichthyologists, or by other American writers, some of whose papers will be referred to later. If text-figure 3 (their drawing) be compared with figure 1, plate 1 (my photograph), the following differences are observable: the pectorals are more sharply pointed, the ventrals more widely spread, longer, and more rounded than in my specimens. The spots are larger and fewer in number (especially on the head and the edges of the pectorals) than in Beaufort and Key West specimens. The most striking difference, however, is to be found in the absence from the latter fish of the dark, roughly parallel lines, which extend from the dorsum of the former out over the pectorals.

I have elsewhere recorded (Gudger, 1910) the difficulties I had in identifying my first specimen as *A. narinari*, but it may not be out of place to repeat them here. The lines above referred to are sometimes present in the living or at any rate in the freshly killed fish, but are so indistinct as to be found only after close search. So nearly invisible are they that my first specimen, examined while alive and in brilliant sunshine, showed no traces of them. Some hours later, when this fish was brought to the laboratory and compared with Jordan and Evermann's figure, despite its agreement with the characters given on pp. 87 and 88 of vol. I of their "Fishes of North and Middle America," I thought it to be a new species; and it was not until Mr. Henry D. Aller, director of the Laboratory, pointed out the very faint striations, that I was convinced to the contrary. It is significant that Jordan and Evermann make no reference whatever in their text to such lines. The photograph (fig. I, plate I), made from my fresh fish, shows that they are too indistinct to affect a sensitized plate, even when exposed 60 seconds. Attention may be called here to the sharpness of focus in this photograph, as even the fin rays are shown with marked clearness.

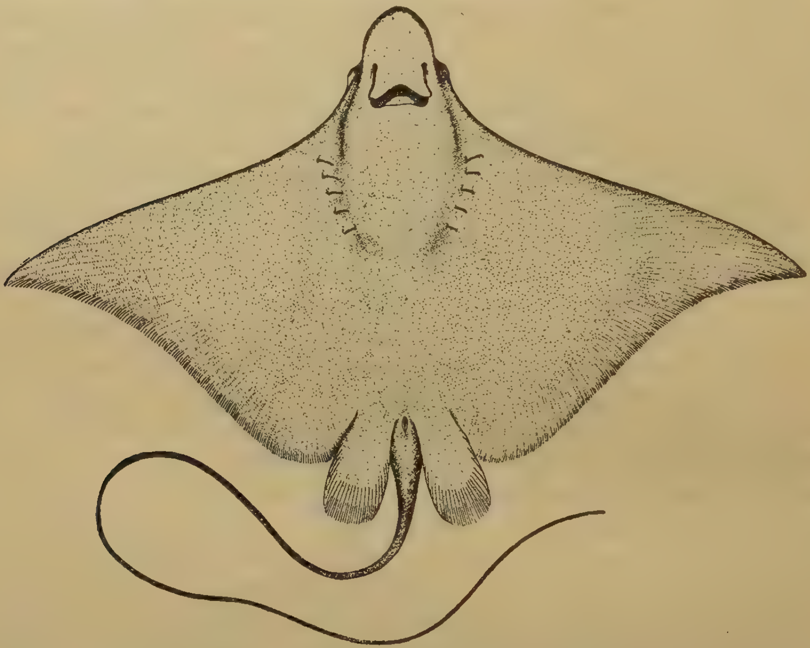
Although the question of the presence or absence of lines on the dorsum of this fish will be taken up at length later, it does not seem out of place here to refer to Abbeville's (1614) *Narinnary* with its back "all striped of black and white." Save for De Laet's (1633) quotation of Abbeville, no writer has referred to these stripes until Smith (1907) wrote: "Color above brown, with numerous small, round, pale spots, and transverse dark lines."

With reference to the fins, another difference between Jordan and Evermann's figure and my photograph should be pointed out. In the former the rays at the angles of the pectorals and on their hinder edges are very prominent. Since the drawing is from an alcoholic specimen, this must be due to the macerating action of the alcohol. In the photograph, which was made from a fresh specimen (dead an hour), such rays are visible only in the ventrals, the posterior edges of the pectorals are finely scalloped, as first noted by Russell (1803), but first shown in the painting in the Royal Library of Berlin and here reproduced as figure 3, plate II. Since particular attention will be given to the head and snout in the section bearing that title, nothing more will be done here than to call attention to the fact that the head is too light in color and too prominent. The spiracles are too marked, the eyes are too prominent and placed too high on the sides of the head, while the snout is too short and too blunt.

Figure 2, plate I, is a photograph of the ventral surface of the fish previously described, and, with the exception of Jordan and Evermann's and Coles's (1913) figures herein reproduced, is the only one known to the writer. Leaving out of further account the various general features already spoken of, in comparing the two figures, no essential differences are noticeable save in the head.

The snout in the photograph shows to better advantage than in the dorsal view, since it is in the ventral plane of the body. That in Jordan and Evermann's figure is plainly too short and rounded. The mouth is too squarely cut, the lower lip especially so. In the photograph the nasal flaps are longer and fit more closely in the corners of the mouth. Still more marked is the sharper angle of the point of the lower jaw and its projection beyond the mouth and indeed over the lower lip. These structures were found in every fish examined by the writer.

The first and only description of our spotted sting ray from Brazilian waters since Marcgrave (1648) is that by Miranda Ribeiro (1907), 259 years later. His very clear diagnosis of the characters, both generic and specific, agrees closely with Jordan and Evermann. These Brazilian rays, however, are dark olive above with blue-white spots, which are round, more or less equidistant from each other, and in size about equal to that of the eyes.



TEXT-FIG. 4.—Ventral view of *A. narinari*, after Jordan and Evermann, 1900, Florida.

As Gunther's (1870) characterization of *A. narinari* from specimens in the British Museum is the most authoritative since Müller and Henle (1841), so his description of the single specimen in the Museum Godeffroy at Hamburg (1910) is the most satisfactory since Jordan and Evermann's (1896). His specimen from Samoa is, he says, not to be distinguished from Atlantic specimens. However, he describes its nose as blunt, whereas my figures and observations plainly show it to be sharp-pointed in all, although of varying widths towards the base. The whole upper surface of the body in his young specimen was covered with numerous bluish-white spots.

In connection with notes on the embryos of certain rays (*A. narinari* especially) taken at Cape Lookout in 1912, Coles has (1913) incidentally published some elegant figures of our ray, which, through his kindness and that of the American Museum of Natural History, are reproduced herein as figures 9, 10, and 11, plate V. One text-figure of his paper will be omitted, since it shows no points not given in figure 9, plate V. This figure is a lateral view of a female 7 feet 2 inches wide. Figures 10 and 11 are head-on and ventral views of the same fish. Figure 10 is the first head-on view ever published, and the only ventral representation except the Jordan and Evermann drawing, (text-fig. 4) is figure 11. Unfortunately this last is taken at too flat an angle to give a clear representation of the whole ventral surface. Further attention will be called to these figures in other sections.[1]

The following table gives in comparative form the measurements (in inches), absolute and relative, of the various specimens of the spotted eagle ray described by the authors previously quoted:

Author.	Length.	Breadth.	Tail.	$B=Lx$	$T=Lx$
Abbeville	72	72	72	1 ±	1 ±
Marcgrave	18	46	51	2.6	2.8
Euphrasen*	....	....	....	....	3
Bloch & Schneider	....	....	....	....	3
Ruppell	....	....	....	2	4
Russell	10	34	62	3.4	6.2
Müller & Henle	12	28	52	2.3	4.3
Forster	11	20.5	35	1.9	3.2
Cantor	7.25	11.75	33.75	1.6	4.7
Bleeker	....	....	....	2 −	3 +
Duméril	....	....	....	2	3 to 4
Klunzinger	....	....	....	1.67	3 to 3.5
Day	....	....	....	....	3 to 4
Jordan & Evermann	....	....	....	2	3 to 4
Beaufort, 1901	26	48	56	1.84	2.2
Gudger, 1909	18	26	40.5	1.4	2.3
Coles, 1909	16	23.5	35.5	1.5	2.2
Gudger (Beaufort, 1910), I	19	28.5	33	1.5	1.7
II	18.5	27.5	39.5	1.5	2.1
III	26.5	37	27.8	1.4	1
Coles, 1910	36	69	69	1.9	1.9
Jenkins	15.5	31 +	32.5	2 +	2
Miranda Ribeiro	....	....	....	2	3 to 4
Gunther	11	....	43	....	4
Coles (Cape Lookout, 1912) I	....	86	....	....	....
II	....	91	....	....	....
III	....	83	....	....	....
Gudger (Key West, 1913) I	24.5	33	37.75	1.3	1.6
II	23	32	44.5	1.4	1.9
III	24	34	60	1.4	2.5
IV	32	62	82	1.9	2.6

SPECIFIC DESCRIPTIONS.

COLOR AND MARKINGS.

Aëtobatus narinari shows such marked variations in color of both the general upper surface of the body and of the spots, that it may not be without value to summarize here the work of the various investigations previously cited and to give my rather full observations of the specimens which I have examined.

Marcgrave (1648) says that the upper part of the body is of a steel (*ferreus*) color while "scattered over the entire fish are white spots about the size of a *nummus misnicus*." This would indicate that they are found on the head, though his figure does not so show. However, they are shown on the head in the water-color painting. In his colored figure the upper surface is a dark steel-blue with white spots. Piso (1658) also speaks of its color being blue.

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[1] In an interesting book on fishing in Florida, "The Giant Fish of Florida," J. Turner-Turner (1902) describes the capture of a number of spotted sting rays. His figures, made from photographs, are quite good. His descriptions need not detain us here.

Omitting those who merely copy Marcgrave without ever having seen the fish, we next come to Sloane (1697) whose Jamaican ray was "smooth, blue, marked with white spots" on the dorsum. His slightly fuller description, dated 1725, makes the fish "all blue even in the flesh itself, with white spots on it."

Browne (1756), whose fish came from the same waters, speaks of the middle parts as bluish-mixed (*media cæruleo-miscella*).

Next comes Euphrasen (1790), whose ray (also a West Indian specimen) was a brown above with round white spots, in diameter about the size of a man's thumb. His drawing (fig. 5, plate III) has the spots too large, too few, and entirely lacking on the head. In Walbaum's "Artedi," volume 3, 1792, Euphrasen is quoted substantially as above, as he is also by Schneider (Bloch and Schneider, 1801).

Russell's (1803) ocellated ray had a dark ash-colored upper surface spotted with numerous small, round, white spots edged with black—these, however, being absent from the head.

The question whether or not Quoy and Gaimard's 5-spined ray from Guam is identical with *A. narinari* will be taken up in the section on the species, but for the sake of continuity it seems well to give their notes on the color. This was a dark brown sprinkled with round sky-blue spots.

Ruppell's (1835) Red Sea ray had its whole upper surface of dark olive variegated with white spots. He declares that his ray, of which he gives no figure, is identical with Russell's Eel Tenkee, which is shown in figure 6, plate III.

Müller and Henle (1841) examined twelve specimens from Brazil, the Red Sea, and the Indies, whose coloring was brown interspersed with white spots scattered regularly over the whole dorsal surface save the head only; but they add that the number of these spots was sometimes small.

Forster's toothless ray (Lichtenstein, 1844), which is *Aëtobatus narinari* beyond any doubt, was lead or steel colored above and "scattered over the body" were many round white spots.

The spotted sting ray found in the Malayan waters was, according to Cantor (1849), "Above greenish-olive or greenish-gray; a little behind the occiput and behind the anterior margin of the pectorals appear more or less numerous greenish-white rounded spots edged with black."

Bleeker (1852), who found these rays common throughout the East Indies, describes them as being coppery green above, the back and fins both pectoral and ventral dotted with scattering round pearly-blue spots. No definite statement is made as to their occurrence on the head.

Duméril (1861) has gone into the question of spots more carefully than any other writer. He says, describing the congeneric *A. latirostris*, which Gunther declares to be a mere variation of *A. narinari* and not entitled to specific rank (on this point see page 315 of this article): "On a brownish-black foundation are roundish white spots irregularly scattered and occupying all the upper surface of the animal." Contrasting it with *A. nari-*

nari, he notes that its spots are larger, farther apart, and hence fewer. The average diameter of 50 spots on *A. latirostris* was 8 to 9 mm., of 100 on *narinari* was 4 to 6 mm. (For other specific differences see page 315). These points are clearly brought out by figure 8, plate IV, a photographic reproduction of Duméril's drawing, inspection of which will show spots on the heads of both species.

Day's (1865) Malabar specimen had a grayish-olive back covered behind the head with numerous dirty-white spots with black edges. He unfortunately gives no figures. Duméril says of *A. narinari*, in his "Histoire Naturelle des Poissons" (1865): "General color brown, with small circular spots of whitish-green bordered by black, regularly distributed and varying in number."

In 1867, the distinguished ichthyologist, Dr. Theodore N. Gill, described an *Aëtobatus*, which had been received by the Smithsonian Institution from San Francisco, under the name *A. laticeps*. Its color was bluish black above, interspersed with numerous fairly distinct whitish or yellowish spots, smaller than the eye. These were smaller on the head and larger on the body and behind towards the sides, and were somewhat ocellated on the ventrals. The pectorals were margined with blackish, while on the best Beaufort specimens the margin is whitish. Some of the Key West specimens, however, had both margins. The specific identity of this specimen will be discussed later.

Gunther (1870), from a study of twelve specimens in the British Museum, merely says that the upper surface, whose color is not stated, is generally adorned with numerous round bluish-white spots. He decides that the variations above noted are not specific and puts all forms into one species, *narinari*. Later (1880), he continues the one species and adds that it may be easily recognized by the numerous bluish-white round spots on the dorsum, though reference to his drawing (text-fig. 2) shows that these are lacking on the head and anterior part of the pectorals.

In 1871, Klunzinger redescribed the spotted sting ray of the Red Sea. His specimen was grayish-black on the dorsal surface and everywhere (except on the head) there were round white spots, rather few in number. Ruppell's ray from the same waters, it will be remembered, was dark olive above with white spots all over the dorsum.

Day's (1878) Indian specimens (localities not noted) were grayish-olive, sometimes greenish-olive or leaden-gray, and were generally covered behind the head with dirty-white or bluish spots edged with black. To this description is added the interesting statement that in immature forms the upper parts are a deep lead color and that the spots are hardly visible.

Chronologically Jordan and Gilbert (1882) come next, but as their description is identical with that found in Jordan and Evermann (1896), which will be discussed at length later, consideration of it will be deferred.

In 1895 Henshall described a specimen from the west coast of Florida under the name *Stoasodon narinari*. It was dark brown above and thickly covered with white spots, about half an inch in diameter.

Jordan, in his "Fishes of Sinaloa" (1895), describes an *A. narinari* from the Pacific Coast of Mexico whose "Color is bluish-black with many round yellowish spots scattered equally over the back and ventral fins; spots about as large as eye on back, smaller on head; sometimes two spots run together, forming an elliptical spot [see here fig. 1, plate 1 of this paper]; about 16 spots from eye along anterior margin of pectoral to lateral angle; posterior margin of pectoral very narrowly margined with white [see my fig. 1]; ventral side pearly white."

On page 2753 of volume III (1898) of the "Fishes of North and Middle America," Jordan, over his own signature, repeats the description of his Mazatlan specimens from Sinaloa and adds that there are no noticeable differences between these (sometimes identified as *A. laticeps*) and the West Indian specimens. A careful comparison of his description with the photograph serving as a frontispiece for this paper will show how true this is for Beaufort specimens. Jordan calls particular attention to the differences between specimens from Mazatlan (above) and the original type specimen of *A. laticeps*, whose locality is unknown but which was received from San Francisco. Its color was "bluish-black with numerous rounded yellowish spots on head, smaller than eye, much larger on body, assuming on the pectoral the form of ocelli." (Page 88, vol. I.)

Evermann and Marsh, in their "Fishes of Porto Rico" (1900), describe the life color of a specimen taken at Culebra Island, near Porto Rico: "General color of whole upper surface light chocolate brown, everywhere covered with roundish or oblong pearly or bluish spots or blotches, largest about size of eye, smallest less than half as large; under surface milky white except margin of snout, which is dark gray; tail uniform chocolate brown; iris yellowish gray." They reproduce the figures in Jordan and Evermann above referred to.

Jenkins (1904) found this beautiful ray rather common at Honolulu, but only made a critical examination of one specimen. He describes it as having a blue dorsum covered with very many clearly marked white ocellus-like spots about the size of the eye, the head in front of the spiracles being devoid of spots and therein very unlike the Beaufort specimens.

Calling our ray *Stoasodon narinari*, Jordan and Evermann (1905), in their "Fishes of the Hawaiian Islands," find specimens from Honolulu and Hilo to have exactly the same colors as those from Porto Rico. One in life was bluish-gray on the dorsal surface and slightly darker on the pectorals, while the back was covered with bluish-white spots, of which those in the middle region were largest and those on the edges of the fins smallest, exactly as may be seen in my photograph of one Beaufort specimen.

Jordan's "Guide to the Study of Fishes," volume I (1905), tells us that *Aëtobatus narinari* "is showily colored, brown with yellowish spots."

In collaboration with Seale (1907) Jordan describes one specimen from Cavite, Philippine Islands: "In spirits the color is brownish, the upper surface of the disk covered with pale blue spots. The pale spots are much fainter than in Hawaiian examples. The latter, however, of much larger size." Here the reader's attention is called to Duméril's statement concerning the relative size of the spots.

H. M. Smith (1907), writing of Beaufort specimens, says that the color is brown on the dorsal surface with many round, small, pale spots and with dark parallel lines [not bands] running transversely. He is the first and only writer to refer to the dark bands shown in Jordan and Evermann's drawing (text-fig. 3).

Recalling Marcgrave's (1648) statement, that his ray was iron or steel colored above, with white spots scattered all over the whole dorsal surface (except the head and snout), it is interesting to have Miranda Ribeiro's (1907) description of Brazilian specimens. Writing 259 years after Marcgrave, he says present-day specimens are, "dark olive above with blue-white spots, which are round, more or less equidistant from each other, and of a size approximately that of the eyes."

Gunther's (1910) South Sea specimen from Samoa is expressly declared to be indistinguishable from Atlantic specimens of equal size. No general color is given, but the whole upper surface is thick-set with round bluish-white spots, those on the head, however, being rather few.

The first and only writer who seems to have ever seen the lines or bars was the French Capuchin friar, Claude d'Abbeville (1614). He states briefly and simply that "this fish (the *Narinnary*) is all striped of black and white."

Excepting de Laet (1633), who merely quotes d'Abbeville, no writer from 1614 to 1907 (Smith) makes mention of these curious and interesting color bands and lines. My own observations on Beaufort and Key West specimens are given at length in the following pages, together with the conclusions based thereon.

I have had from Beaufort five specimens, two in 1909 and three in 1910. Elsewhere (Gudger, 1910 and 1912A) these various specimens have been described. In 1909 I had no knowledge of the great variations in color which are found in *Aëtobatus narinari* and unfortunately made no notes thereon, being chiefly occupied with those points wherein these specimens differed so markedly from those of Jordan and Evermann. At this late day, all that can be said is that their color agreed pretty closely with that given by these authors for their specimens. My notes, however, record the fact that in life no lines were visible on specimen No. 1. I was thoroughly convinced of their absence until Director Aller, after the fish had been dead some hours, called my attention to them. They were, however, extremely faint and only visible when the light fell on the fish at a certain angle. The specimen was dissected for internal organs and jaws and thrown away.

Mr. Coles's specimen, No. 2 for 1909, after being in 5 per cent formalin for a year, was carefully examined. Its general color was found to be a light brown overlaid with a bluish or leaden-gray. The spots which were scattered over the whole dorsal surface (head included) were cream-colored or dirty white. None of them had dark rings around them, but nearly all had dim white centers, giving the ocelli-like appearance. These spots were larger on the back and smaller on the head and outer parts of the pectorals, while near the ventral fins a few ran together to make elliptical or dumb-bell-shaped spots. The right pectoral had on its anterior edge 23 spots, on its posterior 25; the left pectoral had in front 20 and behind 27 spots, not all of which were of approximately equal size. The dorsal had on each side a large spot with a dark hinder edge, and at its base another large spot on each side. On either side of the tail, just under the spines, was a white stripe. The bands shown in Jordan and Evermann's drawing (text-fig. 3), which were not visible to the eye when the specimen was brought to the laboratory and which are not to be found in a photograph of this ray made with a very small diaphragm and a long exposure, were at this time visible as very faint lines, having no width, not bands. This was a male, probably immature, having claspers only half an inch long, pointed at the ends, and with very faint grooves.

Specimen No. I, 1910, was taken June 30 in Newport River, about 4 miles from the laboratory. Its measurements, as well as those of Nos. II and III (to be described later), are given in the table on page 261. In bringing it into the laboratory, it was badly handled and sunburned, resulting in a loss of cuticle in places, but the color could be made out fairly satisfactorily.

In the fresh specimen the ground color was a dark chestnut or brown and the spots a rich yellow cream. These spots were found over all the body, but were smaller on the head, while some few in the pectoral region had run together. The fish was put in a boat and partly covered with water. The cream-colored spots gradually became whitish. That part of the fish out of the water turned dark and finally became a velvety black and the spots *blue*. The dark cuticle peeled off during the day, leaving the ground color a dirty brown and the spots white. In the fresh (just dead) specimens no bands could be found, but after death faint lines could be made out by close inspection; these, however, did not cross the spots as shown by Jordan and Evermann (text-fig. 3), but were rather outlined and marked off by them.

After being in 5 per cent formalin for 19 days, this fish showed the following colorations: The body generally was of a light chocolate brown with bluish-gray regions where the sunburn was not bad (the cuticle gave the grayish or lead-blue color); the spots were white varying to creamy white, some with bright white centers, giving the ocellus-like appearance previously reported. As noted in the first specimen, the spots on the head were smaller than these in the mid-pectoral region—the mid-dorsal and outer pectoral regions were too badly mauled to have their spots described. The spots extended in on the ventral surface of the spiracles. On the right pectoral, anterior edge, there were 17 + spots, and on the same surface of the left fin 18 + spots. Since the tips of the fins were badly split, the numbers must be marked +. The dorsal was devoid of spots on and below it. Below the spines there was a white line or stripe on either side. The transverse lines previously referred to were present and pretty definite. They were extremely narrow bands made by dirty white lines with dark edges, due to the ground color. On none of my specimens were they so marked as on this one.

Specimen No. II was taken in the channel connecting the inner and outer harbors, on July 4. When it came in I was very busy with other matters, so it was put in a tank of running salt water and left for two or three hours. When it was got round to, I found to my regret that the cuticle was beginning to slip, so all haste was made to photograph it. This took so much time that no careful notes of the life color could be made. The ventral view (fig. 2, plate I), the front aspect (fig. 16, plate VI), and the lateral view of the head (fig. 12, plate VI) were all made from this fish.

After immersion in formalin (5 per cent) for 15 days, the color of this specimen showed up as follows: general color pearl gray or bluish-gray; where the cuticle had slipped it was a light chocolate brown; the spots, larger on mid-dorsal region and smaller on the head and at the extremities of the pectorals, were a bright white in the center of the disk and elsewhere a dirty white with distinct white centers, giving the familiar eye-like appearance. Sparsely scattered over the whole disk were dumb-bell-shaped markings made by the coalescence of two spots. The dorsal fin had on its hinder half the familiar white spot changed to a stripe, edged with black, and at its base a large spot. Both pectorals had spots as follows: Anterior edge, 20 spots; posterior, 23; and in both a line of very indistinct spots on the crenate hinder edges. The outer and inner edges of the bases of the ventrals were edged with white. White spots were found in the spiracles as in other specimens. The transverse lines were fairly distinct and in some cases they crossed the spots. This was an immature male, with claspers only three-fourths of an inch long.

Spotted sting ray No. III, 1910, was taken, on July 7, within 300 yards of the locality for No. II, by the same fishermen. I reached the scene while it was still flapping on the beach, and, as soon as it was dead, covered it with wet towels and bringing it to the laboratory (a third of a mile away) photographed it while still fresh. Its picture forms the frontispiece to this paper. While fresh its color was a dark chocolate-brown and its spots were cream-colored, some of them turning a faint bluish or greenish-blue. No striations whatever could be found although they were carefully searched for. After 12 days in weak formalin its condition was almost as perfect as when brought in, but its color was now a bluish-gray while the spots were still cream-colored running to white. This ray had the largest spots of any examined at Beaufort by the writer. Smaller on the head where they were arranged in definite rows, they were pretty uniform in size over the rest of the body, and (more than on any other Beaufort fish examined by me) showed a tendency to run together (see fig. 1, plate I).

Attention is called to the spots of extra large size at the base of the ventrals and to their presence in the spiracles. On the anterior edge of the right pectoral fin there were 20 spots, and in the same region on the left 21. It was not easy to count them on the posterior edges, because especially on the right fin they were so mixed up with the line of smaller and fainter ones formed on the extreme edges in the crenations, and further because the inturnings of these fins next to the ventrals had the spots confluent to form white stripes or splotches. The regular arrangement of these spots in very definite rows was noticeable. These spots, which were of very uniform size throughout, were surrounded by faint, dark circles, thus giving the ocellus-like appearance recorded by so many authors. None of them had the whiter centers noted in my other specimens.

The dorsal fin had two large spots at its base and on its hinder surface a white splotch edged with black. Under the spines was a white streak, and below that (in the middle of the side of the tail) a long, pointed, dark streak. Careful examination of this specimen after immersion in formalin showed the presence of faint transverse striations on the mid-pectoral region. However, the only lines visible in the photograph (fig. 1, plate I) are fin rays.

Examination of the photograph of one of Mr. Coles's huge specimens taken in 1912 shows that this ray had on the dorsal fin a large white splotch and back of this a black border. At the base of the fin is another large white splotch, beneath this a long dark streak ending in a backwardly directed point, and lower still the white side of the tail (see fig. 9, plate V).

Attention will now be called to the spots on the dorsal surfaces of these rays. One of these, a male 6 feet 11 inches wide, had, in the region of the bases of the pelvic fins and at the root of the tail, a few irregularly shaped markings formed by the coalesence of two or more spots, but no distinct ocellations. Another, a female 7 feet 7 inches wide, had practically the whole dorsal surface covered with white spots with dark centers. However, it is a third specimen, a female 7 feet 2 inches wide, in which these ocellated spots are most perfectly developed. Figures 9, 10, and 11, plate V, are lateral, frontal, and ventral views of this ray. Study of these figures shows that these eyed spots are formed by two or more spots coalescing to form U or C-shaped spots and that these finally close up to make white rings with dark centers.

There now remain to be described the four Florida specimens. Three of these, it will be remembered, were taken at Key West by Dr. Mayer on May 10 and were preserved by freezing in a large block of ice. On May 29 the first of these, a male 2¾ feet wide, was examined. The epidermis on this specimen was beginning to slip, but those parts still covered by it were a blackish-brown, those from which it had slipped were a lavender gray. The spots were found all over the dorsum, but those on the head, and especially in front of the eyes, were smaller than those on the other parts of the body. In the middle parts of the back and on the wings the spots were larger and many were dumb-bell-shaped. An average spot measured 11 by 13 mm. There were about 27 spots along the front edge of the right pectoral. When the epidermis was pulled off the spots in it were a smoky-gray, those in the underlying skin were cream-colored. No transverse lines were visible either in the epidermis or in the true skin.

Specimen No. 11, for 1913, was also a male, 2⅔ feet wide, and was examined on the same day as the preceding. With the epidermis on its color was a blackish-brown; with this off it was a leaden-gray. The spots were cream-colored with dark centers. In the pulled-off epidermis they were whitish with dark centers, and those in the flesh were of about the same color, but were surrounded with fairly distinct dark-gray circles well marked off from the general lead-gray body color. The spots over the central part of the body ranged in shape and size from round ones having an average diameter of 10 mm. to elliptical ones 10 by 18 mm. Those on the head, between the eyes and extending into the spiracles, were smaller. Those along the front edge of the left pectoral were about 20 in number. No transverse bars were visible.

The third of the rays preserved in ice was not examined until June 7. It was a male and measured 2 feet 10 inches between the tips of its pectorals. Nearly all of the epidermis was gone from this specimen, but those parts still covered by it were blackish, while the others were a lead or steel-gray. In the epidermis the spots were light gray and on the skin whitish with dark centers. Each of the latter spots was surrounded with a dark ring about half as wide as the spot. On the central and hinder parts of the body the spots ran together to form oblong or dumb-bell-shaped spots. On the front edge of the right pectoral about 27 spots could be counted.

Transverse lines were visible—lines not bands. On running my finger along them I found that they were formed by transverse canals in the flesh just under the skin, which could be made to swell with the contained liquid. For the most part these were about an inch apart and traversed both spots and interspaces. On the hinder part of the back they ran into the large longitudinal canals marking off the outside limits of the abdomen.

On the base of the tail a gray streak on each side ran back to the spines. The dorsal was gray in front, black behind, and had a white spot at the base. The tail was white on the sides and underneath back to the end of the last spine; thence black to the tip. However, about a foot behind the dorsal there was a white spot on the right and two on the left side. This I had not noticed on any specimens before.

My fourth Florida specimen was grained in the same locality as the preceding, *i. e.*, off Slaughter-house Point, Key West Harbor, on June 20. I carried it at once to a wharf and noted its color before it was fully dead. It was a full-grown male, 5 feet 2 inches wide. Its life color was a light chocolate brown, darker toward the hinder edges of the fins, and the spots were creamy white with dark centers. On the center of the back and on the hinder parts of the body the spots were in the shape of dumb-bells, figures 3 or 8, letters S, C, and U, and in oblong and roundish markings. All the round spots had dark centers, but no outer circles were noted. However, small round spots were found everywhere among the markings just described. The large round spots are formed, as the fish grows older, by the coalescence of the horns of the U-shaped markings. All sorts of gradations of these could be found. The hinder edges of the fins were fimbriated and were margined with black, having in front of the black edge an ornamentation of spots, figures, and wavy lines.

In the cephalic region the spots were smaller, but covered the whole head, snout, and cephalic fins, and extended into the spiracles, covering both walls and flaps. The dorsal fin was white edged with black, having in front and along each side of the base a broad dark mass, and having a dark bar on each side extending from above in front obliquely backward and downward, dividing the white area into two regions, each of which had a small, round, black spot in it. The under side of the tail was white like the body, and on the sides, about a foot behind the last spine, were a number of white spots as noted on the preceding fish.

The above are all life colors. After exposure to the sun for some time, the skin turned black and the spots became greenish and finally a faint pale blue. No lines nor bands were at any time visible. For a figure of a spotted sting ray showing markings similar to those above noted see figure 9, plate v, from Coles's 1913 paper.

The foregoing diverse facts with regard to the color and markings of *Aëtobatus narinari* may enable us to understand the great differences in color which have been recorded by the authors cited. It would seem that there are four things to be considered in reconciling these differences: (*a*) that there are great variations in the color of the fish; (*b*) whether the fish was alive or dead, and if dead how long; (*c*) whether the fish was fresh or preserved, and if preserved in what and how long; (*d*) the age of the fish as shown by its relative size.

There can be no difference of opinion about the fact of great natural variations in ground color and markings. My own observations on Beaufort fish, made on five specimens (three of them alive) and on Coles's photographs, and on four specimens from Florida, show that considerable diversity exists in our form. That this is true for those in other waters is shown by the quotations previously given from various authors. Further, there can be no doubt that after death the color changes. My living fish were for the most part brown in color, but after death showed a tendency to change color, becoming dark or bluish. Preserved specimens in formalin all turned bluish-gray or lead-colored, this being due to changes in the cuticle, for when this was stripped off the general color was brown. Not enough data have been collected to show what effect age has on the color of the ray, but it is generally the rule that the older fish may be expected to be darker in color.

From the observations made by Mr. Coles and myself, it seems to be the rule that in young fish the markings are regular, but that as the fish grow older the spots become confluent to form various bizarre figures. These are found mainly in the hinder part of the body, where possibly growth is more rapid. Such irregular figures are noticeable on Coles's 1912 specimens (see fig. 9, plate v) and on my largest Florida ray.

Since Annandale's later notes on *A. narinari* (1910) go far toward clearing up the vexed question of color, I can not do better than quote them *in extenso:*

> In Edinburgh and London there are Indian specimens that agree closely with American and South Sea specimens in the British Museum, while an old female from the Madras coast differs in more respects than one from all other specimens I have seen. It appears, however, that if very old and very young individuals, in both of which the spots are obscure or absent, are omitted from consideration, three colour varieties may be distinguished, as follows:
>
> Var. *A*.—Entire dorsal surface of disk including snout, spotted.
>
> Var. *B*.—Spots on the dorsal surface confined to the post-spiracular part of the disk.
>
> Var. *C*.—Spots confluent into short transverse streaks.
>
> Var. *B* is the common variety in the northern parts of the Bay of Bengal, but is by no means confined to Indian seas. Var. *A* is found off the Coromandel and Malabar coasts as well as in the Atlantic and South Pacific; while Var. *C* is probably liable to occur in diverse places as an individual sport.
>
> The large female recently taken by the *Golden Crown* is practically devoid of spots, which appear to have become almost obsolete. Very young individuals are also unspotted; but in them the spots are just beginning to appear.

In this connection it should be noted that every specimen taken thus far by Coles or myself belongs to Annandale's variety *A*, being spotted all over the dorsal surface, *snout included*. So far as I know no Atlantic or Gulf Coast specimen has ever been taken which would belong to variety *B*. Variety *C* at first blush would seem to offer an explanation for the stripes or bands sometimes found on our specimens, but it seems to have streaks only and not spots. In this connection mention should be made of *Myliobatis asperrimus*, a beautiful ray from the Bay of Panama, which has its dorsal surface marked off by 8 to 10 transverse bluish-white bars and by numerous round, white spots. This ray was first described by Jordan and Evermann (1898), but they quoted the then unpublished paper of Gilbert and Starks, which appeared in 1904 under the title "The Fishes of Panama Bay" and which contains an elegant figure of the ray.

I have been able to find but four records for young specimens of *Aëtobatus narinari.* Jordan (1895), in his "Fishes of Sinaloa," says that he has had five "large" specimens, each 15 inches long exclusive of the tail. Seven Beaufort rays run in length 16, 18, 18½, 19, 26, 26½, and 36 inches; while four from Key West measured 23, 24, 24½, and 44 inches. Of these only the last of each set can be called large. Neither Jordan's specimens nor the smaller ones noted above can be so designated, and it is quite certain that all were very young. The males of all my specimens, save the big one from Key West, had very short claspers, indicating immaturity. This one was 5 feet 2 inches wide and had claspers 3¾ inches long. These, however, are considerably shorter than such appendages on a *Dasyatis* ray of the same size.

As corroboratory evidence of the youth of these rays the following facts may be cited. Elsewhere (Gudger 1910) the writer has reported the bringing into the world by Cæsarian operation of two young *Rhinoptera* *bonasus*, 8½ inches long by 13½ wide. Bleeker has in the same way obtained the young of *R. javanica* 20 inches wide. Later (Gudger 1912A) the writer captured by seining young *R. bonasus* only 13 inches long. There is but little difference in size between the adults of these two rays, this, if any, probably being in favor of *Aëtobatus*. If it is agreed that the fish noted in the preceding paragraph are young, then attention is called to the fact that all were normal in coloring, mine all having spots over the *whole* dorsal surface.

Günther (1910) corroborates this by his description of a young male from the South Seas, which measured 11 inches (long presumably) and whose tail was 43 inches long. Its whole upper surface was beset with round spots, a few being found on the head. However, Cantor (1849) had a young specimen from Indian waters which was only 7¼ inches long by 11¾ wide with a 2-spined tail 33¾ inches in length. This was greenish-olive or greenish-gray above and lacked spots on the head and anterior margin of the pectorals. Likewise, Day (1878) writes that in the young of the Indian form, in which the greenish-olive or leaden-gray adults have the body usually covered with numerous dirty-white or bluish spots, the back is of a deep leaden color and the spots are hardly apparent.

Annandale (1910) tells us that the very young specimens of *A. narinari* are either unspotted or have spots just beginning to appear, thus corroborating Cantor and Day. He had two Indian specimens whose respective measurements were: breadth 23.2 and 20.4 cm.; length 13.7 and 12.4 cm.; and whose tails measured 57 and 47.5 cm. long. His fine figure of the larger of these young males is reproduced herein as figure 17, plate VII. It is entirely devoid of spots, whereas both Jordan's smallest specimens and mine also were beautifully marked.

Klunzinger (1871) is the only student of this ray who has obtained an unborn young, and he contents himself with saying that it measured 12 centimeters. At Cape Lookout, in 1912, Coles (1913) was so very fortunate as to witness the giving birth to several young by a female which he had just caught. These young were uniformly spotted all over the body, the spots being fewer in the head region; these may be seen in figures 9 and 11, plate V, while figure 18, plate VII, is a photograph of one of these young taken after some months' preservation in formalin or alcohol. It will be noticed that the spots are much fainter and seemingly fewer in the preserved specimen. This particular little ray was 286 mm. wide, 171 mm. long, while its tail measured 634 mm.

The question of the presence or absence of lines or bands across the dorsal surface of the spotted eagle ray is a most interesting and puzzling one. They are sometimes present, usually absent; they are sometimes bands, generally lines when present at all. In connection with the description of one of the specimens from Florida, an explanation of the lines on its dorsum has been given (page 269). They are due to the presence between the skin and the flesh of canals filled with liquid, possibly blood.

However, some fish have bands, and on the last day of my stay at Beaufort in 1910 I believe I hit upon the proper explanation. In pulling off some of the skin from the back of a formalin specimen, I found that under the bands in the skin there were distinctly marked-off brown stripes in the flesh. It may be that these two explanations are based on two different manifestations of the same phenomenon, namely that these underlying vessels not only mark off and give rise to the transverse lines, but that after death or in preserved specimens the coloring matter from their liquid contents (perhaps the hemoglobin of blood) soaks out, discoloring the overlying flesh and skin, thus causing the formation of bands.

The facts are that in life these lines, stripes, or bands are either wanting or so faint as to be invisible not only to the eye but also to the photographic plate after a long exposure with good light and with a very small diaphragm; whereas after death, and especially after preservation in formalin, these markings are apt to show up fairly clearly. What effect immersion in alcohol would have, can only be conjectured, but it is quite certain that the specimen from which Jordan and Evermann's figure was drawn had for a considerable time been in alcohol. However, Claude d'Abbeville (1614) says that the whole (upper?) body is covered with black and white lines, and his specimen or specimens would hardly have been other than fresh. But on the other hand Mr. Coles says positively that after examining more than 100 fresh specimens at Cape Lookout he has never yet seen one with transverse bars or bands.

Before leaving the question of markings, it should be noted that it has been reported by various authors that the hinder edges of the pectorals in *A. narinari* are margined with white, while by others they are reported to be black. Indeed, the present writer has himself noted both kinds of margins. The explanation for this apparent anomaly seems to be as follows: The fish grows to some extent by additions to the hinder edges of the pectorals. On these edges the spots form fairly regular rows. Consequently at one stage of development of the fish, the pectorals will be margined with white; a little later, as the fish grows, these white margins form spots which apparently move forward and the margin is found to be black or at any rate darkish. In various specimens all such gradations may be found.

During the summer of 1913, Mr. Coles took at Cape Lookout two abnormally colored specimens of *A. narinari*, the data concerning which he has kindly put at my disposal. The first was an albino female 3 feet 4 inches wide, 2 feet 6½ inches long snout to ventrals, and 5 feet 10½ inches over all. Swimming in water 3 feet deep, this fish appeared to be perfectly white, but after capture (in a seine) it was seen to be covered with light olive and brownish-green markings, which became plainer as the body was exposed to the air and sun. Description can not do justice to this beautiful specimen, which is shown in plate VI, figure 12*A*.

On the following day, Mr. Coles took, almost in the same place, another *A. narinari*, which at first seemed to be totally black, but exposure to the light and air revealed the presence of a few small, widely scattered light spots. Since showing me the photograph, Mr. Coles has misplaced both it and the film, and it can not be reproduced here. This fish was a male 4 feet 9 inches wide, 3 feet 2 inches long, and 8 feet 5 inches long over all.

From all the data cited in this section it is clear that the spotted eagle ray is subject to wide variation in color and markings.

TAIL AND SPINES.

On a preceding page there is a table in which the lengths of the tails of the various specimens previously described are given both in absolute measurement and in proportion to the length of the body. Very great variations and discrepancies are met with, and none greater than in the account of the first describer. Abbeville (1614) writes that his fish is 6 feet (*pieds*) wide and as many long and has a tail a fathom (*brasse*) long with a spine about a full foot (*un grand pied*) long, whereupon he unnecessarily adds "and very dangerous."

Marcgrave's specimen had a tail 5 inches thick at the root and 4¼ feet long, armed with two spines 3 inches long, curved like fishhooks, and placed just behind the inch-long dorsal. His figure and the water-color painting from which it was reproduced both have the spines with a single barb on each pointing toward the head.

Piso (1648) says that the spine approaches the form of an arrowhead.

Omitting reference to those older authors, who quote Marcgrave without adding anything to our knowledge, we next take up Sloane's (1725) illuminating description of the tail of the whip ray:

> The tail was six Foot long, black, small and smooth, of which are made Whips, whence the name Whip-Ray, beyond the Pinna at the End of the Body or in the Beginning of the tail lie one, two, or three Inch and half long flat streight Bones or Radij, they are white, serrated with Teeth on both Sides like a Saw, made so as an Arrow that's bearded, to enter the Flesh easily but not to come out without tearing it, they lie one on another on the upper Part of the Tail, where there is a Hollow or Cavity made to receive them like a Sheath, that they may swim with less Impediment, and only use them on Occasion.

If the reader will now turn to figure 7, plate IV, wherein are shown Quoy and Gaimard's five-stinged tail and my Beaufort specimen with four stings, he will see how exactly Sloane has described tail and stings. However, Sloane is in error in placing the spines in a hollow or cavity on the upper part of the tail. In the large dried tail, referred to above and described later, there is no distinct cavity, but there is a flattening with a very slight concave surface due probably to drying. The spines, of course, offer no impediment to the fish's movements.

Save Sloane only, none of the older writers give so good a description of the tail as Euphrasen (1790). His preserved specimen had a tail flat, whip-like, growing smaller from base to tip, three times longer than the body, bearing at its base a small subtriangular dorsal fin. Behind this were two stings, the hinder twice as long as the anterior, both flattened sidewise with their barbs turned backward. His figure (fig. 5, plate III) is not so good as his description; the stings are entirely too long, the shafts too slender, and the barbs too fine and long.

Russell's Eel Tenkee (1803) had a "tail of great length" (5 feet 2 inches long compared to a body width of 2 feet 10 inches), tapering to a fine point, darker in color than the body (true of all the specimens I have examined), bearing a small dorsal fin and behind it a spine. In his drawing, both fin and spine are placed too far forward, between the ventral fins.

Quoy and Gaimard's (1824) 5-spined tail, previously referred to, is reproduced as *A* in figure 7, plate IV; *B* in the same figure is a dried tail presented to me by Mr. W. H. Shelton, of Beaufort. The photograph was made by laying the tail on the plate in the Atlas of the "Voyage of the *Uranie*," and having put a piece of black paper underneath the white spines, a long exposure was made with a very small diaphragm. Of their specimen, which has the largest number of spines on record of any sting ray of any kind, these authors state that "the particular form of its tail, armed with five very long spines barbed and hooked along the edges, leads us to name it the ray of the five spines, *Raia quinqueaculata*." In the section on species, the question whether or not this is an *Aëtobatus* will be taken up. Suffice it to say here that it is undoubtedly a whip-tailed ray and possibly an *Aëtobatus*.

Ruppell's (1835) *Myliobatis eeltenkee*, from the Red Sea, is identical with Russell's Indian ray from which the specific name is taken. Beyond noting that the spine is "robust" and the tail four times the length of the body, there is nothing in his description to detain us.

Müller and Henle's (1841) description of *Aëtobatis narinari* is wonderfully accurate in all points. Of the tail, which is more than three times the length of the body, they say that in the region anterior to the dorsal fin it is triangular in section, while behind the spine it is compressed and has distinct lateral grooves. These structures, with the exception of the lateral grooves, I find present in the 4-spined tail previously referred to; while the triangular form of the base of the tail extends to the end of the last and longest spine. The lateral grooves are found, however, in another dried tail in my possession.

Lichtenstein (1844) tells us that Forster's *Raja edentula* had a very long, attenuate, cylindrical tail, three times the length of the body of the fish; while behind the short dorsal were twin spines hooked and biserrate to the very tip.

When Gunther (1870) made his "Catalogue of the Fishes in the British Museum," he found therein two (dried?) tails, but, beyond noting that one had four and the other five spines, he gives no description of them.

Klunzinger (1871) describes the tail of the spotted sting ray from the Red Sea as thick only at the root, long, whiplash-like, slightly compressed, and smooth. The pointed, saw-toothed spine, which is twice the length of the eye, is situated on the base of the tail just anterior to the hinder edge of the ventral fins. Day (1878) is the only author who notes with Müller and Henle that the base of the tail is triangular as far as the spine.

Jordan and Evermann (1896) say of the eagle rays in general that they have long whiplash tails with a spine behind the dorsal fin, while nothing is said of the spines of *Aëtobatus narinari*. Later, however, Jordan himself, in volume III (1898), says of *A. laticeps* (Gill), which he believes to be identical with *A. narinari*, that its anterior caudal spine equals the length of the base of the dorsal, which in turn is half the length of the second spine. This the present writer has found to be true of every 2-spined tail examined. Euphrasen, however, was the first to note this peculiarity. Miranda Ribeiro (1907) says that the filiform tail may have from one to five spines behind the small dorsal, but Gunther (1910) affirms that one is the rule though there may be more.

The tail of *Aëtobatus narinari* is always armed with one and ordinarily with two or three spines, while the number may rise to four or five as recorded by Gunther (1870) and as shown in figure 7, plate IV, of my best Bieaufort specimen and of Quoy and Gaimard's plate, which has been previously described. My notes on the tail structures of Beaufort specimens are not so full as could be wished for, but the following data are given to supplement what has preceded. My first specimen, taken June 12, 1909, had a tail 40½ inches long, armed with two spines. My second, taken by Coles at Cape Lookout July 3 of the same year, had a tail 35½ inches in length, likewise bearing two spines, the anterior three-fourths of an inch long, the posterior 2⅛ inches.

During 1910, I examined three fine specimens. The first had a 33-inch tail bearing two spines, the first of which measured 1 inch, the second 2⅛. Specimen No. 2 possessed a tail 39½ inches long, bearing only one spine and it but three-fourths of an inch long. This was evidently a regenerating structure, for the faint groove back of the dorsal showed plainly that a spine or spines had been torn out. My best Beaufort specimen for 1910 was No. 3, which came into my hands while yet alive. Its tail bore three spines, the anterior 1¼ inches long, the middle one (nearly torn off in the net) 1¼ inches. the posterior 2 inches in length. This torn-off sting had two roots.

Mr. Coles's (1910) largest spotted ray was 5¾ feet wide, 3 feet in length, and had a tail 5¾ feet long bearing four spines, but these were unfortunately not measured. Dr. R. E. Coker, when director of the Beaufort Laboratory, recorded in 1901 the capture in the outer harbor of a spotted ray 4 feet wide and 2 feet 2 inches long, with a tail 4 feet 8 inches in length. Tail and spines (which have been excised) are preserved in the museum of the station. The short sting is 2¾ inches long and has two short roots. The longer sting measured 4⅞ inches and has a blunted single root which bears evidence of having been cut off with a knife. The tail is black and has a slight ventral keel.

The 4-spined tail, previously referred to and shown in figure 7, plate IV, is, after 2½ years of drying, 4 feet long. Of its four spines the first is 2 inches long, the second (which lacks a fraction of the tip) is 4⅛ inches, the third 5 inches, and the fourth is 6⅛ inches. The base of the tail as far backward as the tip of the second spine is triangular in cross-section. Behind that it is rectangular with the top and bottom planes slightly bowed or arched outward. The tails of Mr. Coles's huge specimens of 1912 (see page 268) each bore four spines. These are shown for one specimen in figure 9, plate V.

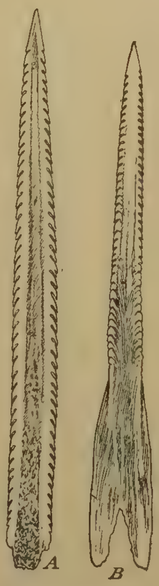
The data given above for the writer's own specimens are from Beaufort rays. Now there will be given similar data for Key West fish taken in 1913. Ray No. I was 2¾ feet wide, 2 feet ½ inch long, and had a tail measuring 3 feet 1¾ inches. Its anterior spine was 1½, its posterior 1¾ inches long.

Ray No. II, 2⅔ feet wide, 1 foot 11 inches long, had a tail 3 feet 8½ inches in length. Its three spines were respectively 1⅝, 2, and 1⅝ inches long. No. III was 2 feet 10 inches in width, 2 feet in length, with a tail measuring 5 feet. Its three spines going from front to back were 1⅞ inches, 2¼, and 1⅞ inches.

Key West ray No. IV was much the finest specimen I have ever had, approximating in size Coles's giant specimens from Cape Lookout. Its width from tip to tip of pectorals was 5 feet 2 inches, length from tip of snout to end of ventrals 3⅔ feet, length of tail only 6 feet 10 inches, length all over 9¾ feet, weight 120 pounds. Like my best Beaufort specimen, it had four spines, but the front spine, torn loose and hanging by a shred of skin, measured only 2 inches; the others were perfectly normal and measured 4⅜, 5, and 4⅜ inches long.

Various authors, notably Jordan and Evermann (1898), give as a specific character of *Aëtobatus narinari* that the second spine is twice the length of the first. Where there are more than two, and especially when there are as many as four or five spines, there is a fairly regular gradation in size. Coles (1910) says that if one spine is torn out the one immediately in front grows larger to take its place. My own observations are confirmatory of this point.

It should be noted here, however, that, perhaps as the result of this multiple spine formation, the largest spine of the spotted ray is uniformly smaller than that in a Stingaree of the same size. The ordinary sting rays, however, not infrequently have two spines, in which case generally, if not always, the anterior sting is the larger. This is true of the several dried tails in the possession of the writer, one of which has four stings.



TEXT-FIG. 5.

Attention is called just here to an interesting difference in the root structure of the spines of these two kinds of rays, which structure has never, so far as the writer knows, been recorded. Text-figure 5 is a photograph of two of these spines: Fig. *A* represents that of *Dasyatis say*, Fig. *B* that of *Aëtobatus narinari*. The sting of *Dasyatis say* which is torn off with difficulty, seems to have no definite bony root, but to be connected with the tough, leathery skin of the tail by a single growth. The spine of *A. narinari*, which is easily torn from the tail, has a bifurcated root in all the Beaufort and Key West specimens examined by me. In confirmation of this point see page 276 for description of the spines of this ray preserved by Dr. Coker in 1901. One has a double root, while the other has had the lower end cut off. The only other figure of such a spine is that of *Myliobatis punctatus*, figured but not described by Maclay and Macleay (1886). This is reproduced here in figure 21, plate VIII. It is bifurcated also, but less markedly so than that of *A. narinari*, *B* in text-figure 5.

The single dorsal fin is situated on the root of the tail just before the spines. The tail under it is triangular in cross-section, the dorsal being situated on the base of the triangle. In the photograph of the 4-spined tail from Beaufort the anterior part of the fin has been cut off, but the posterior portion shows a marking of which I have nowhere found mention and which, being found in every specimen save one (and it in a bad state of preservation) which I have critically examined, seems to be quite constant. This is the white splotch placed more or less vertically on the hinder edge of the dorsal, but having around it always a dark margin.[1] In small specimens this splotch covers the greater portion of the fin, but in old and large fish it is mainly in the upper and hinder portion. Other markings, which I have noted on the tails of all these rays examined carefully, are a white spot (occasionally two) at the base of the dorsal fin, a long white splotch or streak underneath the spines, and below this (on the side of the tail) a long, pointed, dark streak. This is true of Key West rays and of Mr. Coles's huge specimens taken in 1912 (see fig. 9, plate V). Klunzinger (1871) notes that his Red Sea specimen had a tail which was black everywhere save underneath the root, where it was white.

Another curious marking which I have found on tails of both small and large specimens from Beaufort and Key West, results from the presence of irregular, discontinuous dark indentations running vertically on the sides of the tail from mid-dorsal to mid-ventral line, thus giving the tail an appearance which may perhaps be best described as segmented. These indentations are, in some cases at least, opposite each other near the base of the tail, but farther away are placed "staggered," *i. e.*, one on the right placed about half-way between two on the left, and at a considerable distance from each other. At the base of the tail, near the spines, these indentations may be as near as half an inch, but toward the tip the spaces inclosed may be as much as 2 or 3 inches long. On all the Key West specimens these markings are very clear. I find these markings also on the dried tails of another Beaufort Myliobatid, *Rhinoptera bonasus*.

But one record of this has come to light. Jordan and Evermann (1898) in volume III of their "Fishes of North and Middle America," speak of the tail of a Panama ray, *Myliobatis asperrimus*, heretofore referred to, as "crossed by numerous narrow grooves, or indented lines, mostly convex forward, somewhat irregular in position and direction, and not corresponding on the two sides. In the type they follow at an average interval of about 10 mm." See also Gilbert and Starks (1904). This marking may be a family character. It certainly is a curious phenomenon and worthy of further investigation.

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[1] This spot is figured but not referred to in Duméril's *A. latirostris*, fig. 8, plate IV.

The tail of the spotted eagle ray is long, slender, whip-like, and behind the region of the spines is dark in color and often a velvety black. In life it is rounded or but slightly flattened, but dried or preserved specimens, which have been hardened or shrunk by the preservative, are, as previously noted, triangular in cross-section in the region of the dorsal fin and spines, while further back they are rather rectangular in shape with the dorsi-ventral axis about twice as long as the horizontal one. The dorsal and ventral surfaces in dried specimens are slightly curved outward, while the sides are often insunken. At the hinder part of the dorsal flattening, immediately under the spines, there is a (slight) cavity as first noted by Sloane (1725) and by no one else. However, in none of the specimens examined by the writer is it large enough to receive even the one spine immediately over it, much less the whole collection, as Sloane thought. His idea that the ray thus concealed the spines lest they should be an impediment while swimming has of course no foundation. See his statement on page 274.

The ventrally directed apex of the triangular cross-section of the base of the tail forms a kind of keel. This keel extends backward on the ventral side of the tail for a considerable distance as a fairly distinct body, but nothing of the kind has been found on the dorsal surface. The tail of Dr. Coker's specimen, elsewhere referred to, is black and shows a slight ventral keel. This fact has, I believe, not been recorded before.

In the largest Key West specimen the apex of the triangle forms a plainly marked keel on the ventral surface of the root of the tail. This extends in the dried tail in diminishing size backward as far as the tip of the last spine. In this same dried tail on the ventral surface, about the middle of its length, is a very small but plainly perceptible keel. This, however, may possibly be the result of desiccation. In none of these specimens is the tail finned, though we may expect to find an embryonic finfold in the tail of the larval forms.

The table on page 261 records the lengths of the body and tail, absolutely and in relation to each other, for every specimen for which measurements have been given. Ordinarily the tail is said to be 2½ or 3 to 4 times the length of the body (Jordan and Evermann, 1896, and Smith, 1907). The extremes are that the length of tail varies from 1 to 6.2 times body length, the average being 2.9 for 28 specimens, counting in these wide variants. Excluding the extremes, the average tail is 2.7 times the average body in length in 26 specimens. The wide variations recorded in this table are easily explained as follows. It is rather unusual to find a specimen other than a young one with a perfect tail, and the larger and older an eagle ray is the more likely is it to have suffered mutilation in its caudal appendage. Mr. Coles assures me that such has been his experience.

My largest and most perfect specimen from Beaufort had a length of 26.5 inches and a tail measuring but 27.8 inches, the ratio being 1 : 1.05. This tail had plainly suffered amputation, as had the tail of four spines elsewhere referred to. Both were enlarged and bent at the tip, the extreme point being somewhat smaller than the part just anterior to it. Both somewhat resemble fingers amputated beyond the last joint. Only the very young rays have fine slender tails.

My largest Key West specimen, which was a full-grown male, was 3⅔ feet long and possessed a tail 6 feet 10 inches long; the ratio being 1 : 1.9. For so large a ray this one had a very long and slender tail, but about midway of its length there is a very prominent knot, showing that it had been broken but had grown together again. Still further back is a marked bend, showing that it had been injured there also. The tip itself looks as if it had been abbreviated.

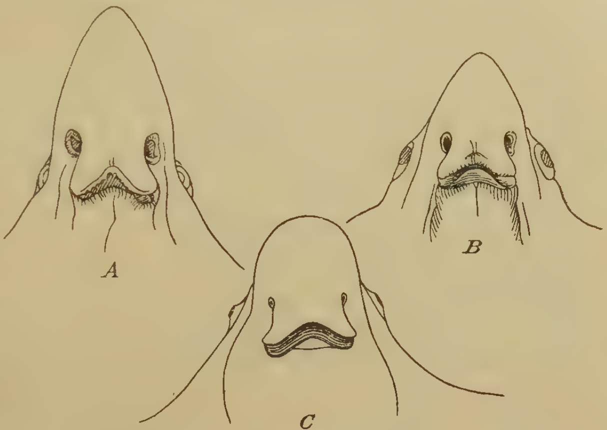
Annandale (1910) found the back and tail of a large female *A. narinari* from the Bay of Bengal to be studded with "small star-shaped denticles. On the head these are sufficiently close together to form a regular pavement, while on the tail they have a spinous character." Nothing of the kind has ever been noticed on any of my specimens, not even on the largest, but Jordan and Evermann (1898), in describing *Myliobatis asperrimus* from Panama Bay, say that it has the greater part of the dorsal surface of the body and tail covered with minute stellate prickles.

HEAD AND SNOUT.

Jordan and Evermann's figures of *Aëtobatus narinari* are possibly the best we have, but compared with those found in this paper they show certain marked differences in the head region. In their drawing the head seems to stand sharply above the level of the body, but the photographic lateral view (fig. 13, plate VI) shows that this is not true, the body in the head region being slightly thinner than it is further back. In the drawing the lower and outer edges of the spiracles are much more strongly marked than in the photographs. In the living fish there is little or no line of demarcation; the skin continues from the dorsal surface of the body into the spiracle without break, as is attested by the fact that spots are found within its cavity. Various figures (on plate VI) show this admirably.

In the drawing the eyes show very prominently; in the photograph (fig. 1, plate I) as in the five living or just dead and the four preserved specimens examined by the writer, they are not visible from above since they are hidden by the lateral projections of the head in front of the spiracles. In Duméril's drawing from the preserved specimen they are barely visible (fig. 8, plate IV).

In the photograph (fig. 1, plate I) the snout is seen to be pointed and of moderate length. Being below the general level of the body region, and the camera being focused on the mid-dorsal region, the head is not shown to best advantage, being somewhat foreshortened. However, the ventral view (fig. 2, plate I) shows the snout in perfect focus. Here it is seen to be long, rather slender, and distinctly sharp-pointed. It is in marked contrast with text-figure 4, which is Jordan and Evermann's ventral view.



TEXT-FIG. 6.—Ventral views of heads of: *A*, *A. flagellum; B*, *A. guttata; C*, *A. narinari.* After Annandale, 1910.

In this connection, it is of interest to contrast with my photographs Annandale's outline figures of the snouts and mouth parts of other eagle rays (text-fig. 6). *A* shows mouth parts and snout of *A. flagellum*, *B* of *A. guttata*, while *C* purports to be a reproduction of Jordan and Evermann's figure, showing these structures in *A. narinari.* However, a comparison of this figure with the original (text-fig. 4) will show how much the artist has erred. If the snout of my figure 2, plate I, were drawn to the same scale, it would be almost as long and slender as *A* in text-figure 6. Figure 25, plate X, is a photographic reproduction of Annandale's elegant figure of *Aëtobatus flagellum.* One's attention is at once called to the notably projecting head and snout, these being strongly marked specific characters for this ray.

The structure of the head and snout is not well brought out in figure I, plate I, but is much clearer in figure 14*A*, plate VI, which is the head only of specimen No. I for 1910, elsewhere described. The head is large and prominent, the spiracles especially so, and the eyes are placed laterally on the sides of the head and are invisible. The snout, however, is of special interest, being long and pointed, quite as much so as is the snout of *A. guttata*, *B* in text-figure 6, according to Annandale. The tendency of the cephalic fins to curl up is noticeable in this figure. The white marks on the head are scratches received in handling and transportation. The fish when caught was perfectly normal. Another type of snout is seen in figure 14*B*, plate VI. This is the head only of specimen No. 2 for 1910. This fish had a snout longer and more pointed than any which the writer has yet seen. In these respects it is comparable to Annandale's number *A* in text-figure 6.

An entirely different type of snout is found in figures 15 and 19, plates VI and VIII. These are photographs of the head of Coles's 1909 specimen. The asymmetry of the snout at once challenges attention, as also does its bluntness and its great width of base. The tending to wrinkle of the loose skin covering the cephalic fins is well shown in the former figure. The other spotted ray taken in 1909 also had an asymmetrical snout and cephalic fins which curled up laterally.

The relative positions of the various parts of the head may be more readily understood by reference to figure 13, plate VI, which is a lateral view of the same fish whose head is shown in figures 15 and 19, plates VI and VIII. Clearly brought out are the lateral positions of both eye and spiracle, and the elongated form of the latter with its valve. This valve is hinged on the roof of the opening in such manner that it swings upward, inward, and backward into a recess to open the spiracle. The semilateral position of the gill-slits, especially of the anterior ones, should be noted. These in the ordinary rays are ventrally located, while the eyes and spiracles are dorsal.

A better view of the head and snout is shown in figure 12, plate VI, a photograph of the same fish shown in figure 1, plate I. It has the pectoral hanging down and the throat is supported on a small box to keep the mouth clear. Attention is particularly called to the color of the snout, the spots on the head, the position of the spiracle at the junction of pectoral fin and head, and the eye with its vertical pupil immediately over the mouth. The snout of Coles's specimen No. 1 for 1912 (fig. 9, plate V) does not show very well. However, the spiracle with its spots is well brought out.

These are the only lateral views of the head of *Aëtobatus* known to the writer. In the course of this research, however, a similar view has been found of the head of another eagle ray. In figure 21, plate VII, is shown the head of Miklouho-Maclay and Macleay's spotted ray, *Myliobatis punctatus* (1886). If comparison of this be made with the preceding figures, marked differences will be seen. The snout is long and slender and curiously upturned at the tip. The spiracle bears the same relative position to the pectoral as in *Aëtobatus*, but the eye is considerably further forward, though still over the mouth.

Figure 16, plate VI, is a "head on" view of the ray lying on a table with the throat supported on a small box to keep the under parts of the head clear of the table. The points of interest shown in this figure are the nasal openings, the color on the snout, the spots on the forehead, the eyes placed laterally with the ball just below the line dividing dorsal and ventral surfaces, the semilateral position of gill-slits, and the size and position of the "wings," the pectoral fins. Above all the figure shows how the ray justifies its name, *Aëtobatus*, eagle ray. Another attractive figure is No. 10, plate V, made from one of Mr. Coles's admirable photographs of his ray No. 1, 1912, taken at Cape Lookout. Attention is called to the relative position of spiracles and eyes, to the sink in the head over the brain, to the white streaks under the eyes, to the dark and spotted nose, and to the spots thickly scattered over the head.

Before leaving the subject, it may be well to refer to the structure of the head and snout shown in various other figures reproduced in this article. Duméril's (1861) elegant figures are shown in figure 8, plate IV. Figure *A* is *A. latirostris; B* is *A. narinari;* while *C* is *A. flagellum.* All these are plainly drawn from preserved specimens, as the shrunken parts show. All three have the median cartilage with the lateral cephalic fins very plain. Very interesting is the gradation in length and width of snouts, and the marked descent from forehead to snout level. The snout of Duméril's *A. narinari* is fully as short as that in my photograph, but much wider. The long, pointed head and snout of *A. flagellum* correspond closely to those same structures in Bloch and Schneider's and to Annandale's figures of the same ray, herein reproduced as figures 24 and 25, plate X.

The head in Gunther's (1880) outline sketch (text-fig. 2) is fairly good. The eyes are out of sight, being covered by the forward prolongations of the base of the pectoral fins. The distance between eyes and spiracles is too great. The snout anterior to the forehead region is too short, and the cephalic fins are too small. It is greatly to be regretted that Forster's (1844) figure of his *Raja edentula* was never published. His description tells us that it had a triangular snout, blunt and flattened, cartilaginous, of medium size, and with edges which showed a tendency to curl or roll up.

Russell's (1803) Eel Tenkee (fig. 6, plate III) had the head too mechanically drawn. The snout is very sharply pointed; the eyes are lateral but too prominent, the fontanelle over the brain is too sharply differentiated from the dorsum, when compared with Beaufort and Key West specimens. Russell says that the snout is soft and that it turns up slightly at the point, The head in Euphrasen's (1790) drawing (fig. 5, plate III) is very poor and unsatisfactory. Both eyes and spiracles are dorsal. The snout is too wide, too short, and lacks the cephalic fins.

Marcgrave's (1648) (text-fig. 1) has the spiracle too far behind the base of the pectoral, and the snout too short, blunt, and rounded. The water-color painting (fig. 3, plate II), from which the figure was reproduced, is considerably better done as to snout, eye, and spiracle; while the oil painting (fig. 4, plate II) has eyes dorsal, spiracles invisible, and snout very long and pointed. The former is an excellent figure, the latter very poor.

In the relative size and shape of the head and snout, as in other structures, the spotted eagle ray shows great variations. In Beaufort specimens we have snouts both symmetrical and unsymmetrical. Two had snouts like those shown in figures 15 and 19, plates VI and VIII, while all the others had equilateral snouts more or less sharply pointed. Duméril's *A. latirostris* (fig. 8, plate IV) is possibly a different species. All the Key West specimens had symmetrical snouts fully as pointed as that shown in fig. 14*B*, plate VI.

JAWS AND TEETH.

The "Portugal" quoted by Purchas (1625), who speaks of —"these Rayes some have in their mouth two bones, and break with them the Wilkes," was certainly referring to Myliobatids, mill-toothed rays, and possibly to our particular form.

However, the earliest definite reference to the singular jaws of this fish is found in Marcgrave's original description. It is worthy of repetition here, for, when it is remembered that Marcgrave studied this ray some 270 years ago on the wild coasts of a new and savage world, one must marvel at the care and minute accuracy with which he did his work. He says:

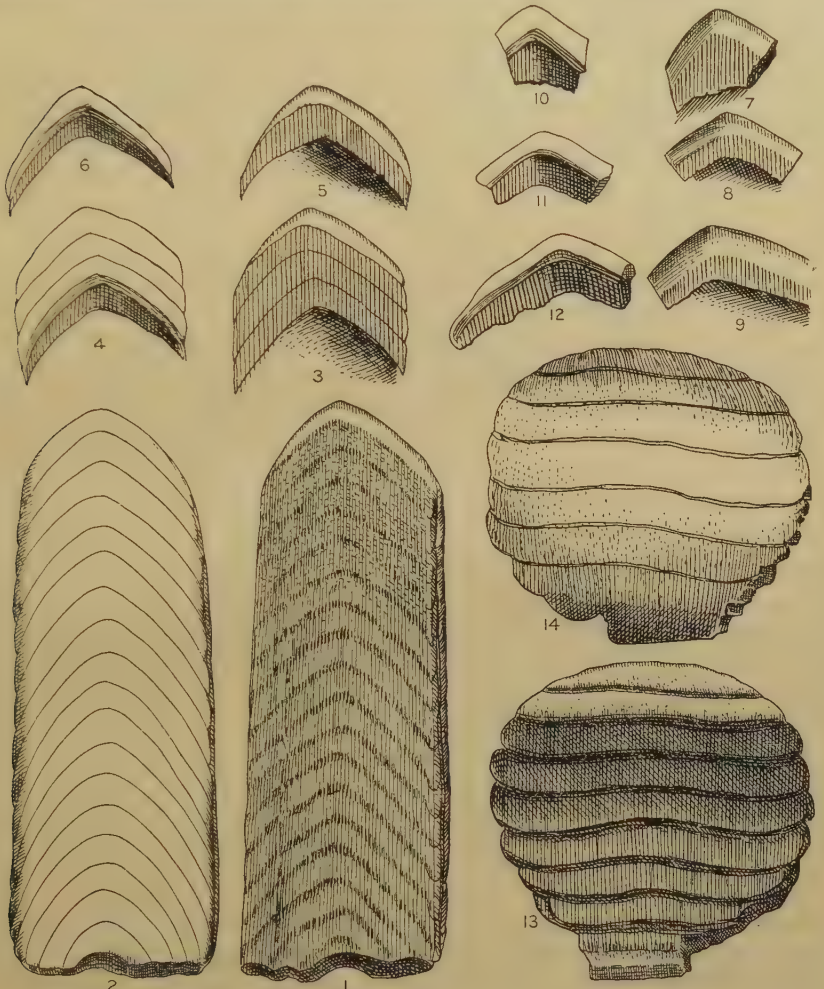
> The mouth is 2½ inches wide, toothless, but having in the place of teeth a lower jaw in the shape of a tongue. This is 4 inches long, 1½ inches wide, and reaches to the external opening of the mouth. Likewise there is an upper jaw placed crosswise, 2 inches long and as many wide. The lower jaw consists of 17 hard white bones having the shape of the letter U and joined firmly to the membranes. Underneath there lie 17 other bones, one under each, of spongy appearance, but not so hard. The upper jaw consists of 14 bones, shaped like the letter J and also joined together by membranes. Likewise there lie above these 14 other bones. Moreover the two jaws are joined to the other bones of the head by membranes [cartilages?].

If the reader will now turn to figures 22 and 23, plate IX, he will see photographs of the remarkable dental armature of this fish. The coincidence may be noted here that the jaws of my 1909 specimen had exactly the number of teeth given by Marcgrave. He speaks of the lower jaw being toothless, but consisting of a tongue-shaped bone 4 inches long, 1½ inches wide, and reaching to the lips. My original notes, made before reading Marcgrave, describe the jaws as being like the slipper-shaped stirrup of a woman's side saddle, the lower jaw being the slipper or bottom part of the stirrup. In illustration see figures 22 and 23, plate IX, of the jaws, and figure 2, plate I, and figure 19, plate VIII, in which the lower jaw is seen projecting from the mouth. Six complete sets of measurements of as many jaws of Beaufort specimens will be given later and four sets for Key West rays.

What Marcgrave meant by his statement that in the upper and under jaws there are other bones, one under each tooth, I am at a loss to understand, unless it be that he refers to the spongy foundation part of each tooth. So Schneider (1801) conjectures when he says that Willughby (whom he seems to have known as a mere copier of Marcgrave) probably refers to the crenate base or some other part subjacent to the teeth for the "under" spoken of in the description. The teeth of this ray are composed of an upper part apparently made of enamel and a lower part seemingly composed of dentine, as will be shown later.

The first man who ever published a figure of the curious jaw structures of *Aëtobatus narinari* was Hans Sloane, whose drawings, from volume XIX of the "Philosophical Transactions," published in the year 1697, are herein reproduced as text-figure 7. Sloane calls this the "tongue" of a flat fish, named *Pastinaca marina*, akin to the Thornback Ray of Great Britain, and found in the waters of Jamaica. He compares this to the lower mandible in man, finds it composed of 19 bones (his figure shows 18) separated by furrows. He confuses top and bottom of the jaw, calling No. 2 the under side when it is the upper, and similarly for No. 1, which is the under side with all the cartilages removed, showing the spongy bases of the teeth previously referred to. Numbers 3 to 12 show very clearly the relation of the enamel and dentine in the individual teeth, and also the shape of the teeth of the lower jaw. Numbers 13 and 14 of this figure are drawn from fossil upper jaws of an Aëtobatid sent Sloane from Maryland. These earliest figures have yet to be improved upon.

The older authors (Willughby, Ruysch, Jonston) either quote Marcgrave or else utterly omit any references to the jaws. Even Euphrasen (1790) contents himself with merely saying: "Mouth below, as in the others of its kind, transverse, with very few and close fitting teeth"; from which we may conclude that his examination was very superficial.



TEXT-FIG. 7.—Teeth of a *Marina pastinaca,* of Jamaica. After Sloane, 1697.

Schneider (Bloch and Schneider, 1801) first quotes Euphrasen as to the teeth, next gives Forster's description (see Lichtenstein, 1844), and then speaks of a figure of the jaws of a *Raja narinari* belonging to Blumenbach in Göttingen. Finally he thus describes a bisected, dried flagellate ray which together with a pair of dried jaws had come into his possession:

> I found in it the same tooth structure as in the free jaws. In the upper jaw there were 8 teeth in the form of the letter V and joined together by membrane; in the lower there were 12, each imitating the letter ζ, all joined together by their crenate bases to the maxilla and occupying its middle part, it being bare on each side.

Next Schneider quotes Willughby (whom he says copies from Marcgrave) and gives the arrangement and kind of teeth correctly for each jaw. This being true, it is inexplicable why he should have attributed the V-shaped teeth to the upper jaw of his specimens, and all the more so since his figure of the under side of the head of *A. flagellum* (the first ever published) shows the long, projecting under jaw with angled teeth (see fig. 24, plate X). Later we shall see that both Agassiz and Owen fell into the same error.

However, Patrick Russell (1803), a physician in the employ of the East India Company, residing at Vizagatapam on the coast of Coromandel, thus speaks of the ocellated ray, *Eel Tenkee* (fig. 6, plate III), which Gunther makes *A. narinari:*

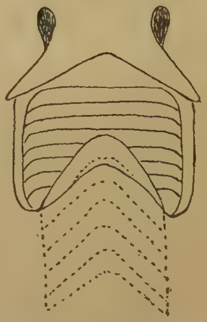
> Jaws dissimilar; the lower arched, narrow, projecting beyond the wider immovable upper jaw; the edges of both are small, without teeth.

He undoubtedly examined the jaws superficially, as his statement "without teeth" would indicate, but this is probably an echo of Willughby, whose book he had and who in turn merely copied Marcgrave.

Blainville (1816), in establishing the genus *Aëtobatus*, contents himself with merely speaking of the teeth as "broad, smooth, polygonal, united, palatine." However, in 1828 when, without any cause discoverable he changed the name to *Aëtobatis*, he goes further: "Teeth large, smooth, polygonal, united into two plates, one lingual, the other palatine."

The first man after Sloane to study the tooth structure of the spotted sting ray was Ruppell, who in 1835 described, from the Red Sea, an identical form or one very closely related to *A. narinari*. Not only did he accurately describe the ray but he both figured and described the teeth. Text-figure 8 is a reproduction of his drawing of the mouth and jaws of *Myliobatis eeltenkee* (synonym for *A. narinari*). He writes:

> The mouth itself is furnished both above and below with a flattened bony mass, whereof that on the upper jaw is subdivided by 6 cross-furrows into 7 rectangular pieces lying one behind the other. The bony plate of the lower jaw extends in front in a sharp angle; it is smaller than the other and like it is divided by furrows running parallel with the outer edge into 7 equal-sized angular pieces lying one behind another.

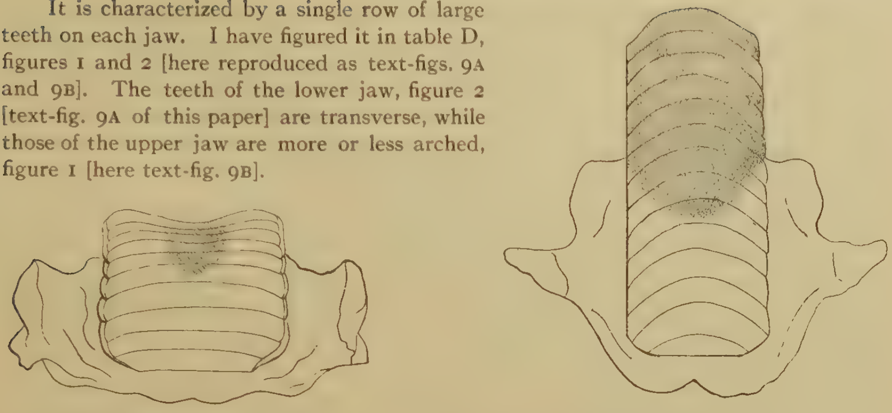


TEXT-FIG. 8.—Jaws of *M. eeltenkee*, from the Red Sea. After Ruppell, 1835.

That this jaw was sketched without removal from the body is plain both from the figure (note the nasal flaps and the dotted lines for the lower teeth) and from the short count. This author was evidently unacquainted with Marcgrave's and Sloane's earlier and more thorough work.

One year later (1836) Louis Agassiz brought out the third volume of his "Recherches sur les Poissons Fossiles." In this volume he deals with the Placoides, and in order to give them their proper setting he briefly takes up the living forms. Of *Raja narinari* or *Aëtobatis narinari* or *Myliobatis narinari* (he uses all these names) he writes as follows:

It is characterized by a single row of large teeth on each jaw. I have figured it in table D, figures 1 and 2 [here reproduced as text-figs. 9A and 9B]. The teeth of the lower jaw, figure 2 [text-fig. 9A of this paper] are transverse, while those of the upper jaw are more or less arched, figure 1 [here text-fig. 9B].



A. Upper jaw (Tab. D, fig. 2). B. Lower jaw (Tab. D, fig. 1).

Text-fig. 9.—Jaws of *Myliobatis* (*Aëtobatis*) *narinari*, after Agassiz.

It seems unbelievable that Agassiz should have allowed to slip into his work so great an error as the above transposition of the jaws, but he did so and he repeats it in the explanation of the plates. Where he says upper, we should read lower, and vice versa. He next goes into a microscopical examination of the tooth structure, into which we will not follow him here.[1] This occurs on pp. 79 and 80 of the general introduction to his "Poissons Fossiles," volume III. However, on pages 325–326 he gives the following illuminating description of the jaws and their functions.

> This genus (*Aëtobatis*) is characterized by the form of the jaws, of which the lower projects in front, while the upper is much shorter and is squarely cut off. Both are furnished with a single row of transverse teeth without lateral chevrons. The lower jaw is, as in the genus *Myliobatis*, longer than the upper jaw (tab. D, fig. 1) [present text-fig. 9B]. The bone of this jaw is longer than wide. The dental plate, whose surface is almost flat in its whole extent, does not cover all the surface of the jaw. In return, its anterior part projects considerably over the jaw, and as the teeth are arched in front, this only makes the anterior edge more salient. Since all the teeth are parallel with each other, their surface offers the aspect of strips curved and joined, the one to the other. The last tooth alone is transversely cut off. The anterior part of the dental plate, which is lightly shaded in figure 2 of table D [present text-fig. 9A], is used for the rubbing of the two jaws against one another. The upper jaw is much wider than long. The dental plate covers it unlike that of the lower jaw, in that the strips are almost straight and only a little bent at their edges, and thus they surround the anterior border of the jaw in such a manner as to form an arched surface in front of the gullet. This part of the dental plate is used for rubbing against the point of the lower jaw.

By comparing these drawings with the figures of the Beaufort specimens, either the photographs (figs. 22 and 23, plate IX) or the outline drawing (text-fig. 14), it will be seen that in the jaws of my fish the lower teeth are much more sharply angled, especially in front; while in the upper jaw the teeth of Agassiz's ray are much more concave than mine. The grinding surfaces are in both sets of jaws practically the same, covering five teeth in the upper and nine in the lower jaw.

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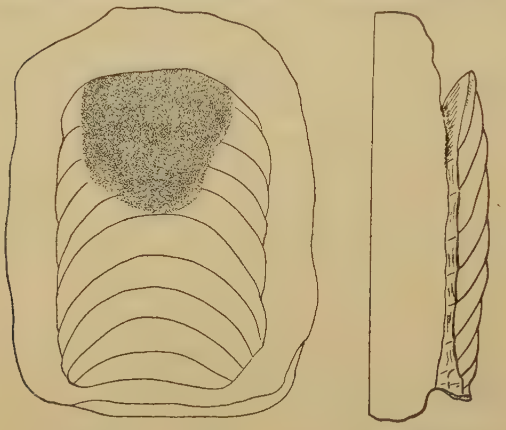
[1] Agassiz notes that the specimen from which these jaws were excised came from the coast of Brazil.

Agassiz records four species of fossil *Aëtobatis* which he had studied, of which he figures only two. Text-figures 10 and 11 are dorsal and lateral views respectively of the lower jaw of *Aëtobatis sulcatus* with ten teeth, the original of which is preserved in the Museum of Paris without indication of its origin.

Müller and Henle (1841), under the heading genus *Aëtobatis*, say:

> The under jaw projects beyond the upper jaw, which has a straight edge. The tooth plates form in each jaw a single row, without lateral teeth, and are in the under jaw bent parallel to the edges of the same. The tooth-plates do not take up the whole width of the jaws.



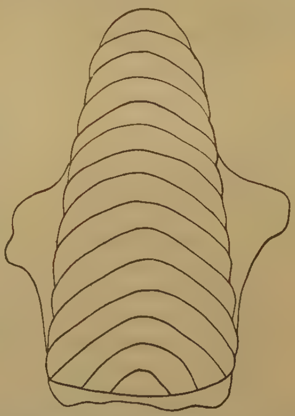
10, Lower jaw. 11, Lateral view of same.

TEXT-FIGS. 10 AND 11.—Jaws of *Aëtobatus sulcatus* (fossil). After Agassiz.

Among the characters for *A. narinari* are:

> The edge of the under jaw and the margins of its pavement teeth present a flat curve which forms in the middle a blunt angle.

Cuvier, in the Atlas to the volume "Poissons" of "Le Regne Animal" (1836–49) on plate 118, figure 4*a*, gives a figure (text-fig. 12) of a lower jaw of which the description reads "Dents de la *Myliobate narinari*." In the text, however, nothing could be found. The rounded outline of these teeth, as compared with the angled structure of Key West and Beaufort specimens, is very noticeable.



TEXT-FIG. 12.—Lower jaw of *Myliobatis narinari*, after Cuvier.

The celebrated anatomist, Richard Owen (1840–45), in his "Odontography," copies both figures and descriptions (including the error) of *Aëtobatis narinari* from Agassiz. This error was for Agassiz merely typographical, of course, and is, as has been shown, corrected in the text, but the same excuse can not be advanced for Owen. Owen comments upon the absence of the lateral teeth found in the Myliobatids (by which he means Aëtobatids of Müller and Henle), upon the strength of the jaws which support and work such heavy teeth, approaching as they do close to the solidity of bone. However, Owen figures a section through the head of a dried *A. narinari*, showing the projecting lower jaw, calls attention to it, and remarks that it can be used in digging shellfish out of sandy bottoms for food. This figure is so small and the structures so imperfectly shown that it has not seemed worth while to reproduce it here.

Forster, whose collections were made 1772 to 1774, but whose work was first made generally known by Lichtenstein in 1844, thus describes the jaw structures of his *Raja edentula:*

> The lower teeth are of bone joined together like a spatula. These [jaws] are formed of many crescent-shaped bones joined together like tiles by a membrane. These teeth are much longer than the upper ones. The upper teeth are also made up of many bones, much broader than the bones of the lower teeth, and are likewise joined together by a membrane.

Cantor (1849) remarks upon the obtuse angle made by the teeth of the lower jaw and specifically states that the teeth are a greenish-white, being of the same color as the spots and approaching the greenish-gray or greenish-olive of the dorsal surface. The teeth of all Key West and Beaufort specimens examined were white. A possible explanation, however, offers itself for this anomalous color of the teeth of Cantor's fish. While at Tortugas during the summer of 1913, I had occasion to preserve a set of jaws in formalin in a copper tank. When they were taken out some weeks later, they had become impregnated with the copper salts and like Cantor's specimen were of a distinct greenish white. It may be that his specimens had suffered a similar impregnation.

Bleeker (1852) remarks that the upper jaw is as wide as long and has 13 teeth, while the lower with 16 plates is twice as long as wide. Day, in his "Fishes of Malabar" (1865), in giving the characters of the genus *Aëtobatis*, by some strange error speaks of hexagonal teeth with small lateral ones, these being the teeth characters of the family Myliobatidæ. However, in speaking of *A. narinari*, he correctly describes the dentition, noting that the lower teeth are obtusely angled.

Duméril (1865) admirably describes the dental apparatus of the genus, and for *A. narinari* notes that the teeth of the lower jaw form a very open curve. This is in marked contrast to the sharply angled teeth of the Beaufort and Key West jaws and of Ruppell's Red Sea form, but is in complete agreement with Agassiz and with Cuvier (see text-figs. 9B and 12).

Gill (1867) records of the Pacific Coast specimen, *A. laticeps* from San Francisco, that its dental plate had the anterior angle obtusely rounded. However, it must be remembered that Jordan thinks this form a mere variant of *A. narinari*.

In Gunther's diagnosis of the genus (1870), in volume 8 of his "Catalogue," from the study of abundant material, consisting of ten specimens, one set of jaws, and two sets of dental laminæ of *Aëtobatis narinari*, he speaks of a single set of teeth in each jaw, the lower projecting beyond the upper. For the species he writes as follows:

> Teeth of the lower jaw are sometimes angularly bent, sometimes nearly straight. Our series of examples shows clearly that this difference is individual and does not constitute a specific character.

Of two half-grown specimens from the Seychelles, one had the lower teeth angularly bent, the other nearly straight; except for these differences they were perfectly identical. The generic characters of the teeth given in the "Introduction" (1880) are identical with those noted above.

Klunzinger (1871) thus redescribes the jaw structures of the Spotted Ray of the Red Sea:

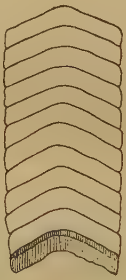
> Upper jaw truncated and broader, but much shorter than the angularly rounded lower jaw which projects from the mouth. The tooth lamellæ of the under jaw form a sometimes pointed, sometimes blunt arched angle in the middle.

In his "Fishes of India" (1878) Day tells us that the teeth of the lower jaw of *A. narinari* may be angularly bent or nearly straight, thus confirming Gunther. He adds, however, that the teeth are greenish yellow. As stated elsewhere the teeth of all Beaufort and Key West specimens examined were white.

Dean (1895), in his "Fishes, Living and Fossil," on page 24, gives a figure (29) with the following legend: "Dental plates of jaw of sting-ray, Trygon (?)." In the text, after referring to the pavement teeth of an ordinary eagle ray (which he also figures), he continues:

> A still more perfect fusion of the dental elements occurs in a ray, closely akin to *Myliobatis;* all lateral elements have been fused, but their metameral sequence has been retained (figure 29).

His figure is plainly that of the jaw of an *Aëtobatus* but unfortunately there is no indication of its source. The commonly accepted idea is that the lateral teeth have disappeared (probably have failed to develop) and that the central teeth have become elongated and arched outwardly. Attention is again called here to Gunther's (1870) series of jaws showing this. Dean's figure is reproduced here as text-figure 13.



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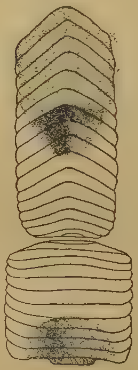

14

TEXT-FIG. 13.—Dental plate of *Trygon*(?), after Dean.

TEXT-FIG. 14.—Teeth of *A. narinari*, from Beaufort, North Carolina.

Jordan and Evermann, in their "Fishes of North and Middle America" (1896) speak of the teeth of the genus as broad, flat, in a single series, upper straight, lower curved, the lower jaw projecting; while for the species they say; "Teeth of the lower jaw straight or more or less angularly bent." Of an Hawaiian specimen, Jenkins (1904) reports the lower teeth to be obtusely angled forward with about five projecting beyond the edge of the upper jaw.

Miranda Ribeiro (1907) thus speaks of the teeth:

> Mouth inferior, with plates of straight prismatic pavement-shaped teeth arranged transversely in parallel rows; truncate in the upper jaw, in the lower the prisms at times folded over to an obtuse angle with points to the front; the plate formed by the latter projects considerably beyond the upper jaw and shows itself over the lower lip.

Compare with this description figures 22 and 23, plate IX. Annandale (1910) finds that the teeth of Indian specimens are transverse and not angularly pointed in the lower jaw.

There are in my possession at this writing six sets of jaws from Beaufort, three of my own taking and three loaned by Mr. Coles. Mine are intact, but Mr. Coles's have the jaws separated and most of the cartilages cut away. Description of these dental apparatuses may not be devoid of interest.

My 1909 specimen was 18 inches long by 26 wide. Its lower jaw (text-fig. 14) is 3¼ inches long by 2 wide, and has 17 teeth of which the anterior 9 are much worn. The upper jaw is 2 inches long by 1¾ wide, and has 13 teeth, of which the 5 anterior forward ones show much signs of wear, the most anterior one having lost the outer third on each side. Owing to the fact that these jaws have been dried flat, *i. e.*, with the upper jaw bent back approximately into the same plane as the lower, I am unable to give the amount of projection of the lower jaw.[1]

Of the 1910 specimens, the first (size 19 by 28½ inches) had a lower jaw 3 inches long and 1 inch wide. It contains 18 teeth, of which 12 show much wear. The upper jaw is 1⅛ inches long by 1⅝ wide, and contains 11 teeth, 5 of which are much worn. The lower jaw projects beyond the upper by 6 teeth or 1 inch. This jaw is viewed from above in figure 23, plate IX.

Specimen No. 2 for 1910, measuring 18½ inches long by 27½ inches wide, being approximately of the same size as the above but having a longer tail, was left as an exhibit in the laboratory and no measurements of its jaws can be given. Ray No. 3, 26½ inches long by 37 wide, was the largest Beaufort specimen I have ever seen. Its lower jaw is 4 inches long and 1⅛ wide, and contains 21 teeth. Of these the 12 forward ones show wear, the most anterior 6 being deeply eroded and the 3 in front having their left edges chipped off. The upper jaw is 2¼ inches long and 1⅝ wide, and contains 15 teeth, of which 6 show hard usage. The lower jaw projects 1⅛ inches or 6 teeth beyond the upper jaw. Figure 22, plate IX, is a lateral view of these jaws showing their relative position when closed.

As noted above, all of Coles's 1910 specimens unfortunately have the jaws separated and cleared of cartilages, and in doing so the soft hinder teeth have been cut away. The size of only one fish can be given. The smallest lower jaw is 3⅝ inches long and 1⅜ wide; it contains 19 teeth, the anterior 8 being badly worn. The upper jaw measures 2 by 1¾ inches; much of the hinder end has been cut off and 4 of its 14 teeth show usage. The second pair measures, for the lower jaw, 3½ by 1⅝ inches. It has 17 teeth remaining, having lost at both ends, for the keen edge on the outermost tooth shows that its predecessor has but lately dropped off. The grinding surface covers the 7 anterior teeth, but at the points of the ninth and tenth teeth there is a slight, irregular depression made by some hard object. The upper jaw of this pair is 2¼ inches long and 1⅞ wide, and of its 13 teeth 5 show much wear.

Coles's largest measured fish was 36 inches long by 69 wide. Its lower jaw, after some considerable abbreviation behind, is still 5¼ inches long, while its width is an even 2 inches. It has 21 teeth remaining, of which the grinding surface extends back over 10, although the next 5 posterior teeth are scarred. The outer right and left thirds of teeth one and two have been broken off. This plate is fully three-eighths of an inch thick, the enamel constituting two-eighths and the dentine one-eighth, but it is very interesting to note that in the outermost and deepest worn tooth the enamel is but one-eighth and the bony portion two-eighths of an inch thick. The upper jaw, which seems to have suffered but little abbreviation, is 3¼ inches long, while its width is 2⅝ inches. It has 16 teeth, 6 of which are in the grinding surface proper, though the next two teeth show rough usage.

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[1] All measurements of jaws are made along the curve and cover or include the teeth only.

It is of course understood that the teeth of this ray, like those of all Elasmobranchs, grow from membrane in the rear and that as they wear out or are broken off in front the growth of those behind keeps the number fairly constant. It must also be made clear that the teeth numbered in the jaws described are the *total* number found by clearing away the overlying fleshy "gum" or membrane. The *apparent* number gotten by counting those visible in the mouth of the fish would be less by some 4 or 5 teeth probably. This fact may be alleged in part proof of Ruppell's (1835) "short" count of the teeth of his ray. He counts 7, but shows 8 teeth in the upper jaw, while in the lower jaw the numbers are 7 and 6 respectively.

The dried jaws of my Key West specimens will now be described. Three of these were rather small, but the fourth was full-grown. All were males.

Ray No. I was 33 inches wide and 24½ long. Its upper jaw is 1⅞ inches long and 1½ wide. It has 17 teeth, of which the forward 6 are deeply worn—practically all the enamel of the outermost tooth having disappeared. The lower jaw measures 3¼ by 1 inches and has 20 teeth; 10 of these show wear, Nos. 6, 7, 8, and 9 are so worn as to be depressed below the general level. Attached to the corners of the outermost tooth are fragments of another, the central and pointed half of which is gone.

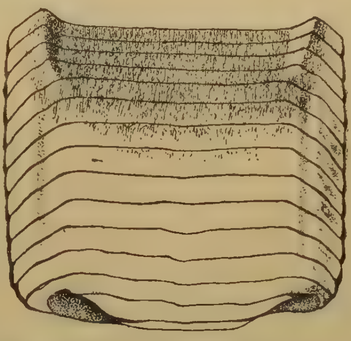
Specimen No. II was 32 inches wide by 23 long. Its upper jaw is 1¾ inches long by 1¾ wide. It has 14 teeth, of which the outer 5 show hard usage, a fragment being broken off the right-hand end of the foremost tooth. The lower jaw is 3¼ inches long by 1 wide. It has 20 teeth, of which the 12 outer show wear, this being greatest in the region of Nos. 4 to 8 inclusive, counting from before backwards. The left third of the first tooth is gone.

My third specimen for 1913 was 34 inches wide and 24 long. Its teeth are the green ones previously referred to. Its upper jaw, composed of 16 teeth, is 2 inches long by 1½ wide. Its 6 foremost teeth are badly worn, the wear stopping abruptly on the seventh. The outer ends of the front tooth are gone. The lower jaw plate, made up of 21 teeth, is 3⅜ inches long and 1⅛ wide. The grinding surface includes the first 10 teeth, and in teeth 6, 7, 8, and 9 there is a marked depression. The outer tooth has the central half only present, the ends having broken away.

Key West ray No. IV was the largest *Aëtobatus* I have ever had and its jaws are in proportion. It was 62 inches wide and 32 long, and weighed 120 pounds. Its upper jaw-plate measures 3⅛ by 2⅝ inches. Of its 16 teeth, the 4 anterior are deeply worn, the enamel of the outermost one being nearly gone. The lower plate is 6 inches long and 2 wide. It has 21 teeth, of which the anterior 10 show much wear, there being a noticeable depression over Nos. 6, 7, 8, and 9.

These teeth-plates being freed from the jaw cartilages, it is easy to see that the lower plate is gently curved with the convexity upward, while the upper plate is sharply bent with the convexity downward. Thus the anterior edge of the upper plate strikes the lower about one-third of the distance back from its point, and for this reason the lower jaw projects beyond the upper by some 5 or 6 teeth generally, and this also accounts for the worn place in the lower plate as noted above (see figs. 22 and 23, plate IX). In all these Florida specimens the lower tooth-plate projects beyond the lower lip by the width of one tooth, and this tooth is free from the underlying cartilages.

In the ten specimens of lower jaws here described and in the other two examined but never excised from the fish, *all* had the teeth V-shaped, as shown in the photograph and the drawing (fig. 23, plate IX, and text-fig. 14); *none* had the teeth "nearly straight," as described by Gunther (1870), or "teeth of the lower jaw straight or more or less angularly bent," according to Jordan and Evermann (1896), or teeth transverse and not pointed in the lower jaw, as found by Annandale (1910) in specimens from the Bay of Bengal, or teeth obtusely rounded in front as shown by Cuvier and noted by others, especially Miranda Ribeiro (1907).

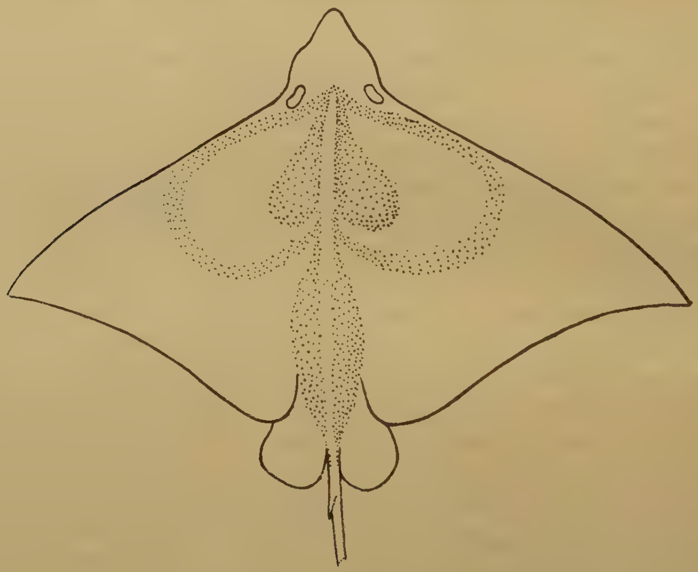

TEXT-FIG. 15.—Upper tooth-plate of an *Aëtobatus narinari*, from Key West, Florida, 1913.

In my specimens not only does the lower jaw project beyond the upper, but each tooth in it projects beyond the next one behind; thus, seen from above, each tooth overlaps the next one in front like shingles or tiles in a roof. The amount of overlapping is certainly equal to and generally somewhat greater than the width of the tooth. It should be noted here that the soft, pulpy teeth at the hinder end of the jaw are slightly wider than the hard bony ones in front. By reference to the figures of the jaws it will be seen that the ends of the upper teeth are curiously bent backwards, giving each individual tooth the form of a very flat bow (text-fig. 15). Careful measurements of every set of teeth in my possession reveal the interesting fact that the length of the upper teeth measured between the points at which the outer bends begin is in every case exactly equal to the width of the lower jaw-plate of that set. Close inspection of these teeth plates shows that in the region of these bends there is a slight depression transverse to the long axis of each tooth. This forms a shallow, longitudinal groove on each side of the upper jaw-plate, and, as noted above, accurately marks off the width and place of contact of the lower jaw. The worn surface of the upper jaw is marked off pretty accurately by these grooves, rarely extending out beyond them more than a mere fraction of an inch. The lines formed by these depressions may be seen if looked for on the smaller specimens, but on the large jaw-plate are very plain, being probably one thirty-second of an inch deep. These points have never been noted before. Text-figure 15 is made from the upper jaw of the largest Florida specimen. In front is shown the worn area, and extending backward on each side in the region of the bends of the teeth are faintly stippled areas marking off this depression.

From a consideration of the data above it will be seen that in the teeth, as in all other structures, the spotted eagle ray is evidently subject to very great variations.

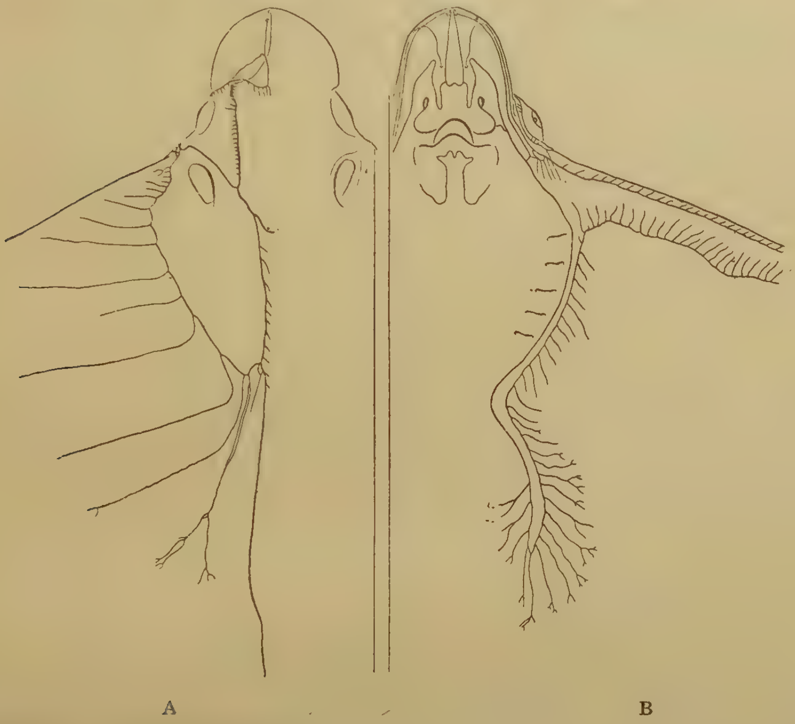
DORSAL SENSORY PORE SYSTEM.

There is now to be described a system of sensory pores on the dorsum of *A. narinari* which has never been noted before, and which I myself overlooked on Beaufort specimens, but found on the frozen Florida rays and also on the live one.



TEXT-FIG. 16.—Dorsal sensory pore system of *A. narinari* from Key West, 1913 (semidiagrammatic).

Beginning back of and between the spiracles and running backward on each side of the median line are fanciful figures made of lines in the flesh, each ending in a black pore. Some distance forward of a line connecting the points of the pectorals, these bands of pores diverge to form two backwardly extending and diverging lobes. However, extending backward between these, on either side of the spinal column, is a band of pores similar to the anterior ones. These widen out over the soft part of the back, dorsal to the abdomen, and further back they nearly converge just in front of the dorsal fin, where they form very distinct patches. From these patches on either side of the dorsal a series of pores extends backward on the sides of the tail, running out to nothing under the spines.



TEXT-FIG. 17.—Lateral-line system of *A. narinari:* A, Dorsal; B, ventral. After Garman.

Beginning at and behind the spiracles a second series of pores extends out along the front edge of each pectoral to about its middle point, then each swings backward, inward, and forward to join the two parallel backwardly going bands just posterior to the lobe-like patches previously referred to. These lines and pores form fanciful figures which run both over and between the spots. So far as my specimens showed, these pores are found rather sparingly on the head, being most abundant on the snout and below the eyes. These pores are presumably homologous with the lateral line pores of Teleosts. Text-figure 16 is a semi-diagrammatic drawing of the dorsal pore-system of *Aëtobatus narinari.* Pores were found on the under side of the body, but not making figures as on the dorsum. The under side of the snout and the central part of the upper lip were so filled with a network of cavities, rather larger than the dorsal pores, as to give the skin over these parts a spongy appearance.

Since this paper went to press, I have chanced upon Garman's memoir (1888–89), "The lateral line system of Selachia and Holocephala." Since his figure and its description are very different from the above, the former is herein reproduced as text-figure 17, A being the dorsal, B the ventral view of the same.

HABITS.

FEEDING HABITS.

Of the spotted sting ray's habits we unfortunately know almost nothing. The fish is too large to be kept in captivity, and its free natural life is such as to preclude anything save scattering observations as to its ways of living.

It is probable that the anonymous "Portugal of Brazil," whom Purchas (1625) quotes in chapter I of volume VII of "His Pilgrims," had reference to our ray when he wrote ". . . . . these Rayes . . . . . have in their mouth two bones, and break with them the Wilkes" whereon they feed. These were undoubtedly pavement-toothed rays, since of all the rays they only live on such fare. But the "two bones" are certainly much more isolated and distinguishable structures in the mouth of an *Aëtobatus* than in that of a *Myliobatis*. Further, our *A. narinari* was common along the coast of Brazil. All these points strengthen the conjecture that *A. narinari* is referred to.

But three of the older writers refer to its feeding habits. Most of them simply copied Marcgrave, while the few who were so fortunate as to examine specimens usually had only preserved material. Furthermore they were systematists, concerned with little else than the characters necessary for classification.

The first of the three is Piso (1648), who in his "De Medicina Brasiliensi," in Book III, bearing the title "De Venenatis & Antidotis," under the sub-heading "Pisces Venenati" writes of *Narinari* that "They are not captured away from the shore. They feed on fishes, for which they lie in ambush and which they capture with the sting of their tail. When they have removed this, they eat them." Here Piso either got his information at second hand or else made a conjecture. Later it will be shown that they live on mollusks alone. However, Piso is correct as to their being shallow-water forms, and it should be noted that this observation of his is the beginning of our knowledge of the habits of the fish.

Sloane (1697) describes the tongue of a ray of Jamaica (plainly *A. narinari*) as made up of 19 bones separated by furrows, and adds that this tongue works against the like bones of the upper jaw to cut, tear, or grind the food; unfortunately he gives no idea of what this food consists. Later, in writing of the same fish, Sloane (1725) says that these rays use their tails "to round their prey to strike them better." In the next paragraph, however, he adds, "They are to be found everywhere in shallow Waters where I was informed they feed on Herbs, Fuci, or Grass." For Sloane's drawing of the teeth of this ray see text-figure 7.

Blainville (1828), speaking of the genus *Raie*, of which he gives *Raies aigles*, *Aëtobatis*, as an example, says:

> The Rays are voracious fishes which feed on other fishes and also on mollusks and crustaceans, which they probably do not catch by swimming, but seize them at the bottom of the seas, in the mud where they have hidden themselves and whence they are able to be dug out by the rays' snouts.

Ruppell (1835) gives a drawing of the jaws (text-fig. 8), the first since Sloane (1725), but it is especially to be regretted that he offers no hint as to the kind of food they are used in grinding.

Agassiz (1836), however, in his figures emphasizes the rough frontal portion of each jaw and explains that this is caused by their rubbing together in feeding; but even he gives no idea of the food of our ray. His figures are reproduced as text-figures 9A and 9B in the present paper.

Owen (1840) figures a longitudinal vertical section through a dried head and jaws of *A. narinari*, comments upon the projecting lower jaw, and conjectures that it "can be used, like a spade, in digging out shellfish, etc., from the sandy bottoms frequented by these rays." Owen is thus the first writer to approximate the food on which this ray lives. He especially calls attention to the strength of the jaws necessary to support such dense and heavy teeth, saying that in density the jaws of this cartilaginous fish approach true bone.

Gunther, in his "Introduction to the Study of Fishes" (1880), remarks of the family Myliobatidæ that the cephalic fins are supposed to be flexible in the living fish and conjectures that they may be used for scooping up food and conveying it from the bottom to the mouth. By implication this statement refers also to the genus *Aëtobatus.* One hardly knows what to think of this. The cephalic fin is soft and the skin-like covering especially so in *narinari;* hence if it is used for the purpose indicated by Gunther, it seems that examination of the living fish ought to show the effects of this hard usage in sand and oyster shells on the snouts. Ordinary sting rays not infrequently are found with bloodshot snouts, but the nine specimens of the spotted sting ray examined by me have shown nothing of the kind. Yet there is no doubt that they feed almost wholly on clams, and Coles (1910) says that they use their snouts for rooting in the sand after the fashion of hogs.

Jordan and Evermann (1896) say of the rays of the family Myliobatidæ that they feed on mollusks, crushing these with their large grinding teeth. Jordan in his "Guide to the Study of Fishes" (1905) makes a similar statement, adding that these fish are destructive to oysters and clams.

Thurston (1894) confirms Jordan by describing the great damage done to the pearl-oyster banks of Ceylon by these rays, the banks being sometimes almost ruined by them. However, he speaks of dissecting an *A. narinari* and finding its stomach full of sea-weed.

The present writer has elsewhere (Gudger 1910) recorded the fact that the food of *A. narinari* seems to consist wholly of clams. Dissections of three specimens in 1911 (Gudger 1912) confirmed this conclusion. However, in not one of the alimentary tracts of the four specimens examined were any fragments of shells to be found. These observations on its food are confirmed in all respects by dissection of the four Florida specimens.

The most extensive and definite observations on the food and feeding habits of this ray have been reported by Coles (1910) in the paper elsewhere repeatedly referred to. He suggests the name "sea-hog" for our ray, on account of its habit of plowing up mud banks and sand-bars with its snout in its search for clams. He has often found it impossible to harpoon rays thus occupied because of the clouded condition of the water. He says further:

> I have known beds, containing many bushels of planted clams, being attacked by schools of these rays and every clam in them destroyed in less than a week; and on several occasions I have had a pile containing a half bushel or more entirely destroyed during a single tide by one or more of these rays. Clams appear to be almost if not entirely the only food of this ray. I have opened more than 50 specimens and have carefully studied the contents of the stomach and have never found that they contain any other food. I am thoroughly convinced that the shell fish consumed by the American people every year are as nothing to the countless thousands of bushels devoured by this ray and its relatives every year.
>
> The muscular development of the jaws of this fish is truly wonderful. I have found in these rays clams which with their shells on must have weighed more than 3 pounds and to crack which a pressure of perhaps a thousand pounds would be required. And I have found in the stomachs of these rays, on a number of occasions, more than a half gallon of clams with the flesh of each clam less broken than the most expert human clam opener could have turned it out; and the writer has often spread out these clams on a clean board and carefully examined them and found that they were absolutely free from any pieces of broken shells.

OFFENSIVE AND DEFENSIVE HABITS.

The spotted sting ray, so far as the writer knows, is an inoffensive fish. The fisherman of Beaufort, notwithstanding the fact that they call this ray the "devil-fish," do not have the fear of it that they do of the common sting ray, *Dasyatis say*, which they familiarly designate "stinger." This is probably due to two facts. First the spotted ray does not, so far as my observation goes, offer so much resistance when taken; and secondly, its spine (or spines), being situated far up on the root of the tail, is not nearly so dangerous as that of the stingaree, which is found about one-third to one-fourth of the distance from the root to the extremity of the tail. Thus the ordinary sting ray can and not infrequently does inflict a wound by lashing out with its tail (I have seen one in the "bunt" of the seine thus drive its sting in the sides of the boat and break off the tip), while, on the other hand, the spotted sting ray, if the object be removed but a few inches from it, can only strike by throwing its whole body. Wounds inflicted by the stingaree are not uncommon and I am acquainted with several Beaufort fishermen who have thus suffered, but the only person whom I know to have been wounded by the spotted ray is Mr. Coles, whose experience will be referred to later.

The commonly accepted idea among fishermen is that this and other sting rays all have poisonous stings, and perfectly reliable men of my acquaintance at Beaufort have described how they have been stung by stingarees, *Dasyatis say*, and how the hand or foot swelled up and what excruciating pain they suffered and how they were disabled for work for a considerable time thereafter. But so far as I can find by dissection and reading no poison gland is ever found in the spotted sting ray or in the stingaree, though there is a groove on the dorsal side of the spines of both rays extending from the point of insertion of the spine to near its tip.

The spine of either ray being closely set with backwardly pointing serrations, it is much easier to cut the spine off and push it through the wound than to pull it out, as Sloane (1725) has pointed out—and such a procedure has been followed in at least one instance known to the writer. The wound is apt to be considerably lacerated, and into it will be carried many pathogenic bacteria imbedded in the slime with which the spine is covered. Furthermore the slime itself, when taken into the blood, may act as a chemical poison. From this it would seem that the explanation of the inflammation is not hard to find. Various modes of treatment are in vogue at Beaufort for the healing of stingaree wounds, but for the most part poultices are used to bring the inflammation to a "head," so that lancing or natural breaking will bring relief.

The only person whom I know to have been wounded by an *Aëtobatus* is Mr. Coles, who in July 1910, while clearing a net, had the misfortune to have a large spotted ray drive its sting 2 inches or more into his leg. He suffered agonies, but having at hand a syringe he thoroughly washed out the wound with a strong solution of formalin. He reports that the pain ceased at once and the wound healed quickly.

The belief in the hurtful and even poisonous properties of the spine of *Aëtobatus narinari* is found scattered throughout the literature from the very beginning. Thus the first European to describe this ray, Claude d'Abbeville in 1614, tells us that its spine is a full foot long and "very dangerous." Marcgrave says nothing on the subject, but Piso (1648 and 1658) writes very interestingly. He tells us that these rays are dwellers in shallow water, where they feed on fishes for which they lie hidden in ambush and which they transfix with the spine. This, however, is erroneous since they feed only on mollusks. Further, our old writer goes on to say that whenever any land or water animal is struck by this spine it receives a virulent poison and the wounded parts suffer great pain, sometimes sufficient to cause paralysis. Whether or not the spine of the dead fish retains its poisonous properties, Piso was unable to say. The antidote for the poison was a plant called Mangue. Piso elsewhere (1658) figures and describes this plant. From the fact that it lives in swamps, and has many branches converted into roots, and further that the figure shows the fruit to consist of long pendant pods acutely terminate at the free ends, there seems to be no doubt that this is the mangrove. Piso further tells us that the natives successfully apply to the wound the ashes of the calcined spine or the split liver of the fish, adding quaintly: "Thus the fish is seen to have in itself the antidote for its poison."

In his later work (1658), in the section dealing with *Narinari*, Piso remarks that many authors have written about the stings of the sting rays, about their poisons and the antidotes therefor, and their hurtful effects on the sound flesh of fishes. These things he says he has verified and has found one of the worst of the sting rays to be *Narinari pinima*, which is the name he gives our ray.

Next, chronologically comes Sloane (1725) whose excellent description of the tail and spines has been quoted on page 274. He adds:

> 'Tis commonly thought this long Tail is useful to the Fish as an offensive or defensive Weapon, wherewith it may lash anything offending it. Or to round their prey to strike them better . . . . . They are eatable: the stings are cut off as soon as they are taken, lest they should hurt unwary People.

Euphrasen tells us (1790) that, since the wound made by the sting is followed by pain and swelling, the inhabitants of St. Bartholomew think it poisonous. Forster (Lichtenstein 1844) is the last author to tell us that the stings are poisonous. He writes:

> The hooked stings contain poison, and for this reason, when the fish is caught, the stings are at once extracted by the inhabitants of Otaheite.

BREEDING HABITS.

Of the manner of reproduction of the Aëtobatids, we have until recently (Coles 1910, 1913) had little definite information. Piso (1658) discourses in general of reproduction in sting rays by the laying of eggs inclosed in horny shells. He ends his long paragraph on this with a few sentences on their stings and poisons and says that chief among such rays is *Narinari pinima*. However, the connection is not close and the inference not clear.

The commonly accepted idea is that all the pavement-toothed rays are viviparous. This belief is probably based on the statement made by Gunther, in his "The Study of Fishes" (1880) and also in his article on Rays in the ninth edition of the Encyclopedia Britannica (1886), that all the Myliobatids are viviparous, but that the young differ much from the adult forms. Waite (1901) quotes a letter from W. A. Haswell, in which the latter states that all the Australian Myliobatids are viviparous. The other Beaufort Myliobatid, *Rhinoptera bonasus*, I have (1912) proved to be viviparous by obtaining (from a specimen only 24 inches wide) two young, measuring 13½ by 8½ inches, rolled up like sheets of paper. Bleeker (1852), by a similar operation on a female *Rhinoptera javanica* of the East Indies, obtained 2 young, 240‴ and 280‴ wide. Couch (1862) in his British Fishes, states that *M. aquila* is oviparous, the eggs being laid in large purses. He figures and describes a young one obtained from such an egg-case; but this must have been a wrong identification. Jordan and Evermann (1896) say that the Myliobatids are ovoviparous, while Smith (1907) affirms that they are viviparous.

Thompson (Jordan and Thompson, 1905) has observed that at the Tortugas these fish not infrequently swim in pairs in long straight lines near the surface. Unfortunately the time of year is not stated. This is probably a pairing play preliminary to copulation. As early as 1810, Risso noted a similar association in the related form *Cephaloptera massena* of Nice. The male was seen for two days swimming around the net in which the female had been taken, "in order without doubt to search for her," and was finally caught in the same net. Blyth (1861) notes of an Indian sting ray, *Trygon imbricatus*, that: "This species is so very often brought in pairs to the bazar, a male and a female, that I can not help suspecting that it lives in pairs, the two being commonly taken together."

The question of viviparity or oviparity in *Aëtobatus narinari* was, however, settled long ago by Klunzinger (1871), who says of the Red Sea *Aëtobatus narinari* that "the foetus measures 12 centimeters." This fact, however, seems to have been generally overlooked. The method of delivery of the young was observed by Mr. Coles in 1910, while fishing at Cape Lookout. After noting that these rays swim near the surface of the water in large schools (one being estimated by him to contain hundreds of individuals) he goes on to state as follows:

> For a number of years my crew and other deep-sea fishermen have been telling me that in giving birth to its young the female ray leaps high in the air as each young is born, but as this leaping seemed so unnecessary I had questioned their tales. However, on about July 15, 1910, I was suddenly called on deck by two of my crew and then I saw a large female *Aëtobatus narinari* leaping high in the air and falling back into the water within 20 fathoms of the yacht. After she had thus leaped several times, I distinctly saw a young one about 6 or 8 inches wide thrown from the body; and after she had again leaped several times without result, another young one was born, and my men told me that two had been born before I came on deck.

While at anchor in the channel off Boca Grande Cay, Florida, in June 1913, numbers of leaping spotted eagle rays were seen, some quite near the boat. I thought that one gave birth to a young one while leaping, but could not be sure.

The explanation of the leaping is probably to be found in the fact that the sudden leap throws the viscera downwards and, aided by the simultaneous contraction of the muscles of the anterior part of the uterus, expels the young. The principle is the same as that by which we drive the last traces of a liquid from an inverted bottle by sudden jerks. Corroborative of this is the fact that whenever a ray is suspended there is a great tendency for the cloacal parts to protrude.

As to how the fœtuses are nourished *in utero* nothing definite was known until Coles published his 1913 paper. Coles states that the uterus of his specimen shown in figures 9, 10, and 11, plate V, was densely lined with large villi 25 mm. in length. These villi secrete a milk on which the embryos are nourished by absorption through the external gills, and later by intake through the spiracles, as the present writer has shown for the other Beaufort rays, *Rhinoptera bonasus*, *Pteroplatea maclura*, and *Dasyatis say* (Gudger, 1910, 1912A, 1913).

However, as early as 1876 Trois had microscopically studied the uterine structures of the related *Myliobatis noctula* of the Mediterranean. He found the walls of its uterus so crowded with villi that the mucosa could not be seen; the number of these, however, decreased towards the mouth of the uterus, which was plicated. These thread-like organs were permeated with blood-vessels, thus enormously increasing the vascularized surface of the uterus. The young were enwrapped by these villi, which, being of great numbers, acted as a feeding organ for the embryos, establishing thus an "efficacious connection between the maternal uterus and the embryo, corresponding to the placenta in *Mustelus acanthias*."

Since writing the above, the following data have come to light from Cuvier and Duvernoy's "Leçons d'Anatomie Comparée" (1846). They say:

> In the eagle-ray, *Narinari*, there is developed only one oviduct, the left . . . . . of which the first part has a round opening, and is folded lengthwise throughout its whole extent. . . . . The second part . . . . . has walls extremely thick and in great part glandular. The inner surface to a depth of 3 to 4 millimeters is composed of interlacing filaments, forming an irregular mesh. Then comes a compact glandular layer, almost a centimeter thick, in which are distinguishable parallel tubes, running straight from interior to exterior. This glandular part is enveloped in a muscular layer clothed in peritoneum.

Mr. Coles states in his recent paper (1913) that, in the female *A. narinari* which gave birth to the four young above referred to, one uterus was much larger than the other. Dr. Louis Hussakof, of the American Museum of Natural History, at my request kindly examined this specimen, which has been deposited in the Museum, and writes that the left oviduct only is enlarged and villous, "the right being very small in comparison and with the villous layer undeveloped."

The young of Coles's ray above noted, four in number, were delivered rolled up lengthwise, as is common in viviparous rays, but died quickly. The various figures of plate v, but especially No. 11, show these young shortly after birth, while figure 18, plate vii, is a photograph of one of these taken after some months' preservation in formalin or alcohol. On comparing this young with an adult, it will be noted that its snout is shorter, its forehead steeper, its eye and spiracle relatively larger, and especially that its spots are fewer, particularly on the head. This specimen measured 286 mm. across the disk, was 171 mm. long, and the length of its tail was 634 mm. These young were spotted even on the head to some extent, but Annandale's young specimen (not an embryo) of this ray was totally devoid of spots (see fig. 17, plate vii).

In connection with the breeding habits, attention is called to the following table showing the length and width of the fish and the length of the claspers in all the males taken by me:

Beaufort.				Key West.			
Specimen No.	Length, inches.	Width, inches.	Claspers, length of, inches.	Specimen No.	Length, inches.	Width, inches.	Claspers, length of, inches.
No. I....	16	23½	0.5	No. I.....	24½	33	0.8
No. II....	18½	27½	.8	No. II....	23	32	.6
No. III...	26½	37	.9	No. III...	24	34	.6
				No. IV....	44	62	3.8

From this table the conclusion is readily drawn that only the last specimen was sexually mature. Furthermore, comparison of these rays shows that, size for size, males of *Dasyatis say* and *hastata* have much smaller claspers than *Pteroplatea maclura*. Only two other naturalists seem to have noted this interesting point. Ruppell (1835) remarks that these organs are small in his Red Sea specimen, and he therefore concludes that is a young one. Bleeker (1852) says that "the genital appendages are short, conical, and non-valvate." Unfortunately neither author gives the size of his fish. The other common Beaufort Myliobatid, *Rhinoptera bonasus*, likewise has very short claspers. None which I have taken had such appendages as long as an inch. Furthermore Mr. Coles states that all the huge males of *A. narinari* that he has taken at Cape Lookout had claspers shorter than in rays of the same relative size belonging to the genus *Dasyatis*.

HABITAT.

The spotted sting ray, *Aëtobatus narinari*, is cosmopolitan in the tropical and semitropical waters of the world. First found on the coast of Brazil by Abbeville in 1614 and by Marcgrave and Piso in 1648, it has since been reported from the same waters by Agassiz (1836), Müller and Henle (1841), Jordan and Evermann (1898), and lastly by Miranda Ribeiro (1907), 293 years after the French friar above named published his book.

Next it is heard of in the West Indies, where it was taken in Jamaican waters by Sloane (1697 and 1725) and by Browne (1756). Euphrasen's specimen came from St. Bartholomew, one of the lesser Antilles, but he gave as its more extended habitat the West Indies and especially those east of the Caribbean Sea. Forster collected his *Raja edentula* in the South Seas somewhere between 1772 and 1776, but his manuscript was not published until 1844. Thus it came about that, from the time of Abbeville in 1614 till that of Russell in 1804 (when he described his ocellated raja from the Indian Ocean), a space of 190 years, it was thought that this ray was peculiar to the tropical waters of the western Atlantic.

Of later references to its occurrence in Gulf-Caribbean waters, there is no dearth. Henshall (1895) found it on the west coast of Florida, and Thompson (Jordan and Thompson 1905) 10 years later collected and studied it at the Tortugas. While in Porto Rican waters its presence has been recorded by Poey (1881), by Stahl (1883), and lastly by Evermann and Marsh (1900), in their exhaustive study of the fishes of that island. Finally Jordan (1887) and Jordan and Evermann (1896) indicate that it is a common form throughout the West Indies. From personal observation and from inquiry among reliable persons I am assured that it is common throughout the Florida Keys from Tortugas to the mainland.

Drifting north with the Gulf Stream in summer, its presence has been recorded at Beaufort by Yarrow (1877), Jordan and Gilbert (1879), Jordan (1887), Jordan and Evermann (1896), Smith (1907), Gudger (1910, 1912A, 1913), and by Coles (1910, 1913). It should be noted that Coles, fishing at Cape Lookout (12 miles from Beaufort), has seen larger numbers and larger specimens of this ray than any other student of the fish. He captured over 50 specimens and saw great schools estimated to contain hundreds of individuals.

Specimens from the Red Sea have been described by Ruppell (1835), by Müller and Henle (1841), and by Klunzinger (1871). The first reported capture of this fish in the Indian Ocean, and, except for Forster's practically unknown collections from the South Pacific, the first from any waters other than the western Atlantic, was Russell's spotted ray identical with *A. narinari* on the Coromandel coast of India in 1803. Among the specimens on which Müller and Henle (1841) based their description was one or more from Indian waters. Day (1865) first collected it on the Malabar coast, and later (1878) reported it as common in all the northern waters of the Indian Ocean from the Red Sea to the Straits of Malacca, but especially in the estuaries of India. While Gunther (1880) found in the collections of the British Museum two half-grown specimens from the Seychelles, a group of islands north of Madagascar. From this data we see that it is widely scattered throughout the Indian Ocean.

Nevertheless, Annandale, the latest writer on Indian rays, in 1909 expressed the opinion that *A. narinari* is restricted to the Atlantic Ocean, and that the Indian forms are to be classed as *A. flagellum* and *A. guttata.* However, in a later paper (1910), he abandons this conclusion and finds that *A. narinari* occurs in the Indian Ocean in three color varieties. For his illuminating description of these see quotation on page 271.

Found in the Malayan waters as recorded by Cantor (1849) and by Day (1878) as previously noted, Bleeker (1852) says that it is common throughout the whole of the East Indian Archipelago, as his lists of fishes for a dozen islands show. However, for the Australian coast but one record has been found. J. Douglas-Ogilby (1886) notes its capture at Cape Hawke, New South Wales.

It is in the Pacific, as is to be expected, that we find *Aëtobatus narinari* most widely distributed. If it is agreed that Quoy and Gaimard's 5-spined ray is a synonym of our fish, then the first capture of *A. narinari* is reported from Guam, one of the Ladrones, in 1824. It is true, however, that Schneider (Bloch and Schneider 1801) had quoted from Forster's manuscript that the latter had taken and described our ray under the name *Raja edentula* at Otaheite, one of the Paumotu group, in the years 1772–1774.

More captures of this ray have been reported from the Sandwich Islands than from any other group in the Pacific. In 1858, Agassiz proposed the name *Goniobatis meleagris* for a specimen sent him from this region. The name was never adopted and the specimen seems to have been lost. It is presumed that this ray is to be identified as *A. narinari*, since no other ray of the kind has ever been collected there, and Jenkins (1904) and Jordan and Evermann (1905) so agree. In 1901, Steindachner identified as *A. narinari* a spotted ray collected by Schauinsland in 1896–97 at Laysan, one of the extreme northwestern islands of the group. On the taking over of these islands by the United States, their aquatic resources were admirably worked up by the United States Bureau of Fisheries. Fishes are reported on in two papers. Jenkins (1904) lists our ray (making it a synonym for *Goniobatis meleagris*) as common at Honolulu, being for sale in the market, while Jordan and Evermann (1905) procured it at both Honolulu and Hilo.

Elsewhere in the Pacific, Jordan and Seale (1907) have taken it at Cavite, Philippine Islands, and Gunther has described a young male from Samoa preserved in the Museum Godeffroy at Hamburg. In the same article he notes that there is on deposit in the British Museum a pair of large tooth plates collected in the Solomon Islands by Woodford.

In 1867 Gill received from San Francisco a spotted ray from an unknown habitat; to which he gave the name *A. laticeps*. Jordan (1895) finds rays all along the Pacific coast from the Gulf of California to Panama practically identical with Gill's ray and with West Indian specimens of *A. narinari*. Later (Jordan and Evermann 1898) he advises that Gill's specific name be dropped and the ray be considered as *A. narinari*.

Gilbert and Starks (1904) report *A. narinari* as frequently seen in the Bay of Panama and note that the specimens they examined were in no particulars different from those described by Jordan from Sinaloa (1895).

From the above data it will be seen that the habitat of this ray is coexistent with the tropical and semitropical waters which encircle the globe.

ECONOMIC VALUE.

Marcgrave's statement that the flesh of *A. narinari* has a good flavor and is sufficient (in the case of his or some other large specimen) to feed 40 men indicates that this ray among others was eaten in Brazil even at that early day. Sloane (1725) declares it to be eatable, and Browne (1756) says it was well liked by the Jamaicans, while Forster (the specific name of whose ray is *edentula*) some fifty years later found it "not the last fish" at the feasts of the Otaheitans. Further Euphrasen (1790) affirms that its flesh is white and, when properly prepared, that it is equal to that of the best fish eaten in Sweden, from which we may conclude that it was habitually eaten in the Lesser Antilles.

On this subject Bleeker (1852) writes: "The flesh of *Aëtobatis narinari* is of a rather dark bluish color and in Batavia, especially among the Chinese, it is much sought for and commands a high price." Day (1878) notes that this ray is eaten by the natives of Hindustan. Jenkins (1904) found it for sale in the market at Honolulu, from which we may infer that it is habitually eaten in the Sandwich Islands. Probably fuller investigations will show that it is a common article of food throughout the Pacific.

Since great numbers of other and more edible fishes are to be had for the catching or are for sale at exceedingly low prices, neither *A. narinari* nor any of the other rays found at Beaufort or Key West are used for food there. However, it is stated by some sailors and deep-sea fishermen, that the pectoral fins of the rays (kinds not specified) are, when properly prepared, excellent for the table. As to this, the writer is unable to speak from experience, but he has eaten the fins of the common southern stingaree, *Dasyatis hastata*, and found them very palatable.

GENERA AND SPECIES OF ANGLE-TOOTHED RAYS.

The observations of the present writer having been restricted to one form, *Aëtobatus narinari*, and that in but two localities, he hesitates to go into this subject further than to collect here the data brought together in the historical part of this research. It is certain that the fish is subject to great variation, and it is possible that the great differences, especially in color and in the presence or absence of spots on various parts of the body, are, as Gunther (1870) found for the teeth, varietal and not specific. If many specimens from the various tropical waters could be collected and compared by such taxonomists as Gill, Jordan, Evermann, Boulenger, Smith, or Regan, it might be possible to reconcile the many discrepancies revealed in the various descriptions quoted in this paper. Descriptions are at best very unsatisfactory, while figures, being for the most part made from (more or less badly) preserved specimens, are but little less so. What is needed is to have these various rays side by side that critical examination and careful measurements of minute details of structure might be made. But these fishes are so large and unwieldy, and their preservation for these reasons so difficult, that such an examination, however much to be wished for, is of the very distant future.

There are, however, at least two and possibly three or four well-marked species which seem to have gained places for themselves in ichthyological literature. But at least one generic and several specific names have been given which do not seem to have had permanence. One species has been pretty firmly established, but is attributed to an author who merely copied from another copier. Other Myliobatids have by some been made synonymous with *A. narinari*, when they belong to entirely different genera. The facts will be herein presented and the reader may form his own conclusions independently of these expressed by the present writer.

AËTOBATUS NARINARI.

GENUINE SYNONYMS.

The species known longest and best is *Aëtobatus narinari*, the history of whose nomenclature will now be briefly sketched. The name *narinari* (in its various spellings) appears to have been a Brazilian or Indian word meaning sting ray. It seems to have been first applied to the ray bearing that name by Abbeville in 1614, but the name and the ray were together first given a place in scientific literature by Marcgrave in 1648. Euphrasen (1790) first designated it binomially as *Raja narinari*, wisely retaining the native name, and to him is due the credit for having assigned the specific designation. For the specific characters see descriptions by Marcgrave and Euphrasen, pages 246 and 249.

However proper the specific term, Euphrasen's generic name was entirely too indefinite, so Blainville in 1816 established the genus *Aëtobatus*, with the following characters:

Body fleshy with pectoral fins shaped like an eagle's wings; head thick, not beaked (*non rostrata*), furnished with a simple appendage in front; eyes lateral; teeth broad, smooth, polygonal, united, palatine; pectoral fins pointed; anterior margin convex, posterior concave; pelvic fins small, round, whole; dorsal fin single on the root of a tail at times extremely long, flagelliform, armed with a serrate spine, without fin at extremity.

Following this is a list of species, ten in all, of which *narinari* is one.

In 1828, Blainville, in "Faune Française" under the heading "Poissons Cartilagineux," without assigning any reason whatever therefor, writes the generic name *Aëtobatis* and repeats almost verbatim the generic characters given above. The chief difference is that the teeth are now described as "large, smooth, polygonal, united into two plates, one lingual, the other palatine," but in neither set of characters are the teeth of the lower plate described as bent or angled with the projection forward. The generic term *Aëtobatus*, having been assigned with definite and correctly designating characters twelve years previously, is the correct name for the genus and is accordingly so used throughout this paper. It should be noted here that *narinari* is not named as a species under this new genus *Aëtobatis*.

Müller and Henle (1841), in their epoch-making "Systematische Beschreibung der Plagiostomen," once and for all gave our ray a definite place in ichthyological literature as *Aëtobatis narinari*. Their description is elsewhere (page 251) given and need not be repeated here, but it should be noted that, among the generic characters, they say of the teeth that the under jaw projects beyond the upper jaw, which has a straight edge, while the under jaw has the teeth bent parallel to the edge of the same; the teeth plates in each jaw form a single row without lateral teeth and do not occupy the whole breadth of the jaw, while for *A. narinari* it is stated that the lower teeth are in the form of a flat arch (*flache Bogen*). They are the first authors to base their description on abundant material, since they had at hand 12 specimens, 6 dry and as many in alcohol, from such widely different regions as the waters of Brazil, the Red Sea, and the East Indies. The great pity is that, not knowing of Blainville's early name, they should have assigned the later generic term which has unfortunately been used down to the present time.

In working up the synonymy of *Aëtobatus narinari*, it is now necessary to return and to identify certain rays described in the interval between Euphrasen's assigning the specific name and Müller and Henle's firmly establishing our ray as a distinct form.

Schneider (Bloch and Schneider 1801) adopted Euphrasen's nomenclature, *Raja narinari*, and although he copied Euphrasen, Forster, and others, he expressly says that he had a hitherto undescribed specimen. Russell's (1803) *Raja ocellata*, Eel Tenkee of the natives, is plainly an *A. narinari*, as figure 6, plate III, shows. So likewise is Raffles's (1830) *Myliobatis narinari*, which is expressly identified by Bennett with *Raja narinari* Euphrasen and with Eel Tenkee Russell and is thought (erroneously) to be the same as Shaw's *Raja guttata*.

In 1835, Ruppell described his *Myliobatis eeltenkee* from the Red Sea. This ray is, as his figure of the teeth (text-fig. 8) shows, an *Aëtobatus*, and his description as surely makes it *narinari*. Cuvier (1817) gives in the atlas of the volume "Poissons" of his "Regne Animal" a figure (see text-fig. 12) of the teeth of *Myliobatis narinari*, but his text contains no reference whatever to it.

As noted elsewhere, Forster collected in the South Seas in 1772–74 a ray which he called *Raja edentula*. His manuscript, published in 1844, contains a careful description which plainly makes his ray identical with the fish being studied. It has, however, been generally overlooked by authors. Blainville (1816) possibly refers to it when he gives *fosteri* as one of his ten species of *Aëtobatus*. Cuvier, in "Regne Animal" (edition of 1817), lists *Raja narinari*, quoting Marcgrave's *narinari* as a synonym. In a later edition of the same work he gives the name *Myliobatis narinari*. In neither case does he give any description. It should be noted, however, that the earlier reference is in a footnote to the genus *Myliobatis*.

Notwithstanding the lack of spots on the head and anterior edges of the pectorals, there seems to be little doubt that Cantor's (1849) *Stoasodon* (porch or projecting tooth) *narinari* is a synonym of our fish. Indeed his giving it the specific name indicates this. Why he introduced a new generic term is unknown to the writer. Following the lead of Müller and Henle, Bleeker (1852) uses *Aëtobatis narinari*, as does Day (1865), though Day's ray lacked the spots on the head.

Five years later Gunther (1870), from the wealth of material in the British Museum, consisting of ten specimens, two multi-spined tails, one set of large jaws, and two sets of excised teeth, made one species, *Aëtobatis narinari*, and this notwithstanding the fact that the "teeth of the lower jaw are sometimes angularly bent, sometimes nearly straight." Gunther declares that these form such a series that he is satisfied that the differences are entirely individual and in no wise specific. Further he makes all the preceding forms (except *Raja edentula*, which seems to have been unknown to him) synonymous with *Aëtobatis narinari*.[1] This decision of this great systematist, based upon the most abundant material since Müller and Henle's, and resulting in the adoption of their nomenclature and its repetition in his "Guide to the Study of Fishes," authoritatively fixed the name until this day.

Accordingly Klunzinger (1871) named his Red Sea ray *Aëtobatis narinari*, though it lacked the spots on the head. Day (1878) also uses the name for Indian Ocean rays, though they also are devoid of cephalic spots. Jordan and Gilbert (1882) prefer this name, as does Douglas-Ogilby (1886). Henshall (1895), however, reverts to Cantor's nomenclature, *Stoasodon narinari*, for a specimen from the West Coast of Florida.

Jordan (1895) uses for the Pacific coast form the name *Aëtobatus narinari*, which the present writer believes to be correct, while Jordan and Evermann (1896) prefer the same termination for all American rays of this kind. So does Steindachner (1901). Moreover, Evermann and Marsh (1900) and Jenkins (1904) write it *-tus*, as do Gilbert and Starks (1904). Nevertheless, one year later Jordan and Evermann (1905) and Jordan and Thompson (1905) again take up Cantor's generic term *Stoasodon*, retaining *narinari* as the specific name. However, in the same year was published Jordan's great "Guide to the Study of Fishes," in which the name is written *-tus*, while two years later he and Seale name the Philippine form *Stoasodon*.

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[1] Among these are such definite species as the ray called *Raja flagellum* by Bloch and Schneider and *Aëtobatis flagellum* by Müller and Henle, as well as such doubtful ones as *Raja guttata* Shaw, *Raja quinqueaculata* Quoy and Gaimard, to say nothing of such a form as *Goniobatis* (correctly *Myliobatis*) *macroptera* McClelland, which is not even an Aëtobatine. All the data available will be given for these forms in the next few pages.

Miranda Ribeiro (1907), who, if one may judge by his table of synonyms, has gone into the question of synonymy more carefully than any one else, uses the correct name *Aëtobatus*, referring it directly to Blainville's paper of 1816. This name is also used by Coles (1910, 1913). Not so, however, Annandale (1909–1910) and Gunther (1910), since they retain the Müller and Henle terminology. However, all these names seem to refer to the same spotted eagle ray, which, if there is any virtue in priority, must be designated *Aëtobatus narinari*.

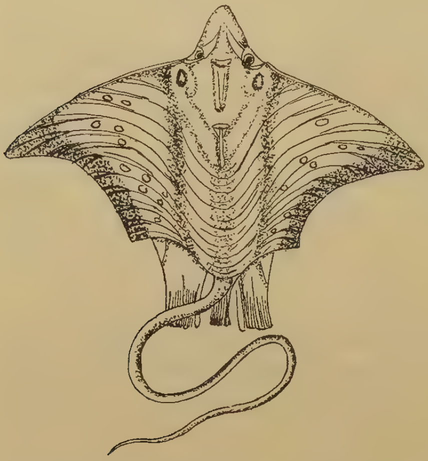
DOUBTFUL SYNONYMS.

It is now necessary to take up the descriptions of certain rays the names of which have been declared by certain authors to be synonymous with the ray originally named by Marcgrave, *Narinari*, but about the correctness of which much doubt exists.

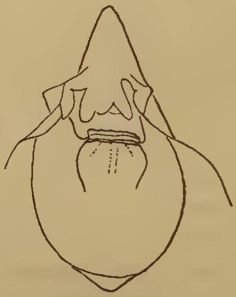
First of these is *Raja guttata*, which is ordinarily ascribed to Shaw, but which was first described by Bloch and Schneider in 1801 in the following words: "Body ashy-gray, black, guttated and spotted, head heart-shaped, tail finned and twice as long as the body." This ray they make identical with Marcgrave's Brazilian ray *Jabebireba* or *Jabebirete*, which, however, is not an eagle ray at all.

Raja guttata, the spotted ray of Shaw (1804), is by many authors identified as *A. narinari*, and Shaw himself says that it is identical with Marcgrave's *Narinari* and with Russell's *Eel Tenkee*. His figure is reproduced herein as figure 20, plate VIII, and his description may be found on page 313 of this paper. Inspection of this figure and comparison with that which forms the frontispiece to this paper, and with any other figure which has been identified in this article with *A. narinari*, will, I believe, show that no allowance for bad drawing can account for the great discrepancies. Later I shall show that this figure is not original with Shaw, but that he copied a mirror image of Lacépède's figure labeled "La Raie Aigle"; and that Lacépède in turn copied an unpublished figure of Commerson's of an eagle ray which he had figured from Madagascar waters. With these facts before him the present writer rejects *Raja guttata* Shaw as a synonym for *A. narinari*.

It is possible that Quoy and Gaimard's (1824) *Raja quinqueaculata* may, as Jordan and Evermann (1896) and many other taxonomists think, be identified with *A. narinari*. The points in favor of such identity are: (1) the elongated snout curved at the tip; (2) body above dark brown in color and sprinkled with round blue spots; (3) tail, which "appears to have been cut off by accident," with a dorsal fin and five spines very similar to those found on my best Beaufort specimen. Their figure of the fish was lost, and, what is most of all to be regretted, they omit any description of those structures, the teeth, which would have forever settled the question of identification. At first the present writer was inclined to admit the identity, but further study has brought out certain objections.



TEXT-FIG. 18.—*Myliobatis macroptera*, after McClelland, India.



TEXT-FIG. 19.—Ventral view of head region of same.

Quoy and Gaimard preserved the tail and deposited it in the Museum (of Paris?) and in their plates give a figure of it. A photograph of this drawing and of my 4-spined Beaufort specimen is shown in figure 7, plate IV. It is very noticeable that the large white spot found on the upper and back part of the dorsal of *A. narinari* is absent from that of the 5-spined ray. In all the dorsals of both Beaufort and Key West specimens examined by me, it has been present save in one only, and it a badly preserved one. This objection alone is, however, not of sufficient weight to negative the identity, but in the course of this research the present writer chanced upon a description by Miklouho-Maclay and Macleay (1886) of a spotted ray, *Myliobatis punctatus*, from the Admiralty and Hermit Islands. This ray also had: (1) an elongated snout turned up at the tip; (2) the upper surface (greenish-gray) dotted with (irregularly scattered dirty-white) spots; (3) a short tail with a dorsal followed by two serrated spines. So far the descriptions are quite parallel, and the figures of this latter ray (No. 21, plate VIII) might well be taken for the former, but these authors go on to describe what the others do not, the teeth which are in "many longitudinal rows." To the present writer it seems quite as easy to identify the 5-spined ray as *Myliobatis punctatus* as *A. narinari*, and because of these doubts he prefers to omit it from his table of synonyms.

Jordan and Evermann (1896), probably quoting Gunther (1870), include among their synonyms *Goniobatis macroptera* McClelland. On looking up McClelland's paper (1840), one finds that he describes and figures, under the name *Myliobatis macroptera,* an eagle ray from the Indian Ocean. Text-figures 18 and 19 are reproductions of his drawings, the latter of the under side of the head. If the figure be inspected carefully, it will be seen that the dorsally placed eyes, the short, round-pointed pectorals forming posteriorly a rounded projection where the tail joins the body, the broad-based, square-ended ventrals, which McClelland says equal one-third the entire length of the body, the short tail, the roughly parallel lines extending across the disk and bent backwards over the body proper, and the few scattered spots on the pectorals only are not found in any figure or description of *A. narinari* published by any other writer. Then if text-figure 19 be studied, it will be seen that the lower jaw is square-cut instead of angled outwards. Further McClelland compares his ray with *M. maculatus*, notes that it has rounded ventrals, whereas his ray has angular fins, and ends by declaring that his ray is an entirely new form. These points clearly show that *Myliobatis macroptera* can by no means be considered as identical with *A. narinari.*

In a verbal communication to the Boston Society of Natural History, June 16, 1858, Agassiz (1859) proposed, on the basis of a specimen sent from the Sandwich Islands, a new genus, *Goniobatis*, for angle-toothed rays having the palate broadest behind and with plates obtusely angular having the rounded edges forward. This description exactly fits Cuvier's drawing of the teeth of *Myliobatis narinari* (see text-fig. 12). For the specific name, Agassiz used the term *meleagris* (guinea fowl), probably in allusion to the spots. Neither term seems ever to have been used, and Jenkins (1904) considers the name synonymous with *A. narinari.* This is probably correct, but as the fish, so far as the present writer knows, was never carefully described, we can not be absolutely sure. Agassiz also proposed that the *A. flagellum* of the Indian Ocean be renamed *Goniobatis flagellum*, but his suggestion was never taken up.

In 1867, Dr. Theodore N. Gill described, under the name *Aëtobatis laticeps*, a spotted ray sent from San Francisco, but of unknown habitat. As the specific name implies, the head was wide and the snout obtusely rounded. Jordan and Evermann (1896) assign it a place in their "Fishes of the North and Middle America" (vol. 1, page 88) and give as its habitat the west coast of Mexico from the Gulf of California to Panama, but mark it as doubtfully separated from *A. narinari.* Gill himself notes that it is closely related to this form and also to *A. latirostris* A. Duméril, and probably not far from Agassiz's *Goniobatis meleagris.* In his "Fishes of Sinaloa" (1895) Jordan states, on the authority of Evermann, that this west coast supposed *A. laticeps* is identical with *A. narinari;* while later (1898), in volume III of the "Fishes," etc., he proposes to omit *A. laticeps* as a species on the ground that no essential differences between it and *A. narinari* can be found.

AËTOBATUS FLAGELLUM.

This ray is the second of the two definitely established species. Under the name *Raja flagellum*, it was first described and figured by Bloch and Schneider (1801). They give the characters as follows: Body width equals twice the length, head and pectoral fins pointed, dorsal fin short, one or two spines serrated on both sides at the base of the whip-lash-like tail, which is four times longer than the body. Habitat: Indian Sea on the coast of Coromandel. A photographic copy of their colored drawing is given as figure 24, plate X. This unfortunately does not show the parallel striations running from front to back across the pectoral fins. It must be noted that in their diagnosis of the characters they say nothing of the teeth, but in the smaller figure, showing the under parts of the head, the projecting lower jaw with its rounded tooth leaves no doubt as to these structures.[1]

Blainville (1816) after giving the characters of the genus *Aëtobatus* (see page 307) enumerates *flagellum* as one of the species under this genus.

Müller and Henle (1841), after examining 21 specimens from India and the Red Sea, established the form *Aëtobatis flagellum*, giving as a synonym *Raja flagellum* Bloch and Schneider. Among its distinctive characters are: Snout wide at base, small, and only about three times as long as the nasal lobes; teeth sharply rounded; no spots; disk above dark violet or bronzy or coppery, white below. Richardson (1846) lists this ray in his "Fishes of China and Japan," but does nothing more than refer to Müller and Henle and to Bloch and Schneider. Likewise Blyth (1861) contents himself with recording it in his "Cartilaginous Fishes of Lower Bengal," but gives no information whatever about it.[2]

The next reference to *A. flagellum*, which has been chanced upon is from A. Duméril (1865). In this paper is a fine plate showing the head of what he calls *A. fouet* (*A. flagellum* Müller and Henle). This elegant plate is reproduced as figure 8, plate IV, of this paper, and *C* is the head referred to. Attention is called to the extremely long head and snout, the latter being very slender and narrow, to the lateral eyes, to the spiracles with their backwardly prolonged depressions, and to the complete absence of spots. It is greatly to be regretted that Duméril gives us no data as to the fish from which the figure was made.

Earlier in this paper (page 309) it has been noted that Gunther (1870) makes *R. flagellum* Bloch and Schneider a synonym of *A. narinari*. This is a palpable error which curiously enough has been repeated by Jordan and Evermann (1896), who probably copied Gunther. The facts are as follows: On page 361 of Bloch and Schneider (1801), under genus *Raja*, No. 10 is *R. flagellum;* lower on the same page, No. 11 is *R. narinari*.

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[1] In his 'Analecten für Vergleichende Anatomie," sammlung I, published at Bonn in 1835, A. F. J. C. Mayer describes *Raja fasciata* Mas." This ray was 6 inches long with a 35-inch tail. Of it he says: "Both the upper and the lower jaw is each overlaid with a tooth-plate consisting of about seven horse-shoe shaped lamellæ. . . . The side fins are striped and are cut in a half-moon shape." This might lead one to think that he had an eagle ray like Bloch and Schneider's *Raja flagellum*, but he says further, "Head square (viereckig)."

[2] Agassiz's (1859) attempt to rename this ray *Goniobatis flagellum* has been previously referred to.

Having made no careful search for references to *A. flagellum*, it is quite likely that I have overlooked a number. However, the only other one which has been found is in Annandale's "Batoidei of the Indian Ocean" (1909). This author considers this ray rare in the upper part of the Bay of Bengal, since he has seen but two examples and a head. However, he gives an elegant figure, reproduced herein as figure 25, plate X. Especial attention is called to the extreme length of the head and snout, to the lateral eyes, and to the widely spread ventrals with their claspers free only at their very extremities, as in young Beaufort and Key West specimens.

Text-figure 6 is also reproduced from Annandale. *A* in this is a ventral view of head and mouth parts of *A. flagellum*. The lower jaw in this ray does not seem to project so much as in the other Aëtobatines, notably in *A. narinari*, as shown in figure 1, plate I. *B* and *C* are ventral views of the heads of *A. guttata* and *A. narinari* respectively. The latter purports to have been copied from Jordan and Evermann (1898), but as has been shown elsewhere is very defective. The former ray will now be briefly discussed.

AËTOBATUS GUTTATA.

The history of this ray, the third of the definitely established species, is very obscure, but the facts seem to be as follows: Under the name *Raja guttata* it was first described by Bloch and Schneider in 1801. Their brief and imperfect description may be found on page 309 of this paper. The species seems to have been given a definite place in ichthyological literature by Shaw (1804), whose description reads as follows:

> Greatly allied to *Raja aquila* in appearance, but with a more produced head or snout; color above deep cinereous, pretty thickly marked with small, round, white or whitish spots; tail fins and spines placed nearer the body than in the preceding [*Raja aquila* with an ordinary sting ray (*Dasyatis*) tail and spine], of which, however, it has sometimes been considered a variety rather than as truly distinct; a native of the Indian and African seas; observed by Commerson about the coasts of Madagascar, by Dr. Russel about those of Coromandel, and long ago by Marcgrave about those of Brazil.

Shaw gives a figure of this ray, which on investigation I find to be a copy of Lacépède's (1798) drawing of *La Raie Aigle*, figure 2, plate 6, differing from it only in that the tail is shown bent to the left side, *i. e.*, it is a mirror image of Lacépède's figure. However, one wades through the mass of Lacépède's verbiage about an eagle ray found in the Mediterranean without finding any reference to the figure bearing the same title until in the concluding paragraphs the real facts appear.

It seems that there had fallen into Lacépède's hands certain unpublished manuscripts and drawings of the naturalist Commerson. Among these were the figure and description of an eagle ray which he had found in the waters around Madagascar and the Isles of France. This figure Lacépède published and labeled *La Raie Aigle;* Shaw in turn appropriated and published it as *Raja guttata*, without giving Lacépède any credit whatever, and merely mentioning Commerson, as has been shown. Shaw identifies his *Raja guttata* with Russell's *Eel Tenkee* and Marcgrave's *Narinari*, but (as has been shown elsewhere) this is probably incorrect. We are not even sure that it is an *Aëtobatus*, since the teeth are not described. Figure 20, plate VIII, is a photograph of this Shaw-Lacépède-Commerson figure.

The only other reference to this ray which the writer has found is in Annandale's "Batoidei of the Indian Ocean" (1909). This author says that it is very common in the Bay of Bengal. From his three (two young, one mature) specimens, Annandale thus describes its color:

> Dorsal surface of young of a uniform dark slate-gray, without trace of spots. The spots of the disk on the adult are confined to the posterior half. They are of a bluish tint and are edged with a faint greenish halo. Their size varies considerably. The ground color of the back of the adult has, in fresh specimens, a beautiful greenish refulgence.

Text-figure 6, from Annandale, gives the under side of the head of each of the three definitely established Aëtobatines, *A. narinari*, *A. flagellum*, *A. guttata*. The subjoined table, in which certain characters in these three rays are compared, is also from Annandale. The data for *A. narinari* are taken by Annandale from Jordan and Evermann's text and figures (1896 and 1898), that for the other two from fresh specimens of the Indian forms.

It will be noted that Annandale's description agrees with Lacépède's figure quite well. It is to be regretted that he has not given us a more minute description of this ray, whose very existence has been much doubted, and especially that he has not given us a figure. In his paper he does not indicate that *A. narinari* is found in the Indian Ocean, and possibly if the matter were gone into with sufficient care and accuracy it might be found that all these Indian and Pacific Aëtobatines with spots only on the posterior half of the body belong under *A. guttata*.

Character.	*A. guttata.*	*A. narinari.*	*A. flagellum.*
Snout...........	Conical, bluntly pointed, distinctly retroverted, at least as broad at the base as long.	Rounded at the tip, much broader at the base than long, straight (?).	Pointed, straight, much longer than broad at the base.
Coloration of dorsal surface.	Uniform dark slate gray in the young, ornamented with bluish spots, which are confined to the posterior half of the disk in the adult.	The whole disk including the head covered with whitish spots both in the young and the adult.	Disk in the adult of a uniform dark greenish bronze color, without spots.
Size............	Diameter of disk in adult at least 125 cm.	Diameter of disk in adult 51 cm.	Diameter of disk in adult 47 cm.
Habitat.........	Tropical parts of the Indian Ocean.	Both sides of the Atlantic; Gulf of Guinea, American coast as far north as Virginia, West Indies.	Red Sea, Bay of Bengal.

Since the above was written, I have received a copy of Annandale's second paper (1910), which very satisfactorily explains the lack of spots on the anterior parts of *A. narinari*. He is quoted in full on page 271.

AËTOBATUS LATIROSTRIS.

August Duméril in 1861 described from the region of the Gaboon River, west Africa, a new Aëtobatine, *A. latirostris*, differing from *A. narinari* in having a shorter and broader snout, with more pronounced cephalic fins, and fewer, larger, and more widely spaced spots; further, the posterior appendages, tail, and ventral fins are much more markedly separated from each other and from the hinder part of the pectorals. His plate is herein reproduced as figure 8, plate IV. Duméril states that 50 spots on the pectorals averaged 8 to 9 mm. in diameter, while 100 spots from the same region in *A. narinari* averaged 4 to 6 mm.

In 1869, Gunther found a spotted ray from the Bay of Panama to be almost identical with the foregoing, differing from it only in that "the soft rostral appendage is naturally turned upwards like the nose-leaf in certain Chiroptera, and is not stretched forward as represented by M. A. Duméril." This may be true of this form, but fresh Key West and Beaufort specimens have the snout almost straight (see figures in plate VI). Specimens in formalin have the rostral parts somewhat distorted, the snout being more tilted than in nature. Specimens kept in a barrel had the snouts badly distorted by pressure against the sides of the barrel. Gunther's preserved specimen had probably suffered in the same way.

In 1870 volume 8 of Gunther's "Catalogue of the Fishes in the British Museum" appeared and in 1880 his "Guide to the Study of Fishes" was published. In neither is there reference to any Aëtobatine other than *narinari*, *A. latirostris* being made a synonym of *A. narinari*.

Whether or not these rather marked differences are weighty enough to constitute this west African form a separate species, the writer is too little versed in taxonomy to say, and when so great an authority as Gunther pronounces adverse judgment, he can but keep silent. It should be noted, however, that Gill (1867) recognized that his *A. laticeps* is very near to *A. latirostris*, and further let it be remembered that Jordan (1895 and 1898) finally rejects *A. laticeps* as not being specifically different from *A. narinari*.

UNESTABLISHED GENERA AND SPECIES.

It will be recalled that in 1816, Blainville enumerated ten species under the genus *Aëtobatus*. Of these *narinari* and *flagellum* seem to be genuine, while *filicaudatus*, *nichhofii*, *ocellatus*, *fosteri*, etc., seem to fall under other genera of the family Myliobatidæ or may possibly be duplicate names of other Aëtobatines.

In 1823, Temminck published a letter from Van Hasselt, in which the latter speaks of establishing a new species, *Myliobatus cyclura*, very similar to Russell's spotted ray. The language is so obscure and the description so imperfect that I have been able to make out only the fact that Van Hasselt thought that he had a ray differing from Russell's ray only in specific characters. This reference seems to be entirely isolated.

Richardson (1846), in his "Ichthyology of China," lists a ray under this heading "?*Myliobatis oculeus*." His description is taken from an unpublished drawing in Reeves's collection. He is undecided whether this beautifully spotted ray is "a *Myliobatis* or *Aëtobatis* which is perhaps only a variety of *M. maculatus*." He did not see the fish and hence could say nothing of the jaws, the deciding structures. Gunther (1870), however, makes this name synonymous with *M. milous.*

Gill (1893) gives the name *A. tenuicaudatus* for an Australian form, making it synonymous with Hector's *Myliobatis tenuicaudatus.* Reference to Hector (1877), however, throws it out of the genus *Aëtobatus,* since it has teeth in seven rows in each jaw.

Starks and Morris (1907), in their paper on the "Marine Fishes of Southern California," list an *Aëtobatus californicus.* However, Mr. Starks in a recent letter to the present writer, says that this ray does not have angled teeth and that he now calls it *Myliobatis californicus.*

Last of all, Gunther (1910) reverts to the fact that in 1870 he put all Aëtobatines into one species, *A. narinari,* notes that the characters upon which the species are based consist of differences in the length of the snout and in the distribution of the spots, and concludes by saying that he is not yet persuaded that he was in error in 1870, since forms with snouts intermediate between long and short and forms with and without spots are of frequent occurrence.

However, on turning a page we find this author describing, under the name *Aëtobatis punctata,* a spotted ray from the south Pacific synonymous with Miklouho-Maclay and Macleay's *Myliobatis punctatus* (1886). The plate of these latter writers, giving various views of this fish, is reproduced herein as figure 21, plate VIII. If this is not sufficient to throw this ray out of the genus *Aëtobatus* (see mouth in the figures) then let it be recalled that these writers in their text say "Teeth-plates of many longitudinal rows of teeth" (see their drawing). That it is, however, a transitional or intermediate form would seem to follow from the further statement that the middle rows of teeth are largest and that the teeth-plates of the upper jaw are nearly twice as wide as the lower, the proportion being that of 48 to 27.

Snyder, in his "The Fishes of Okinawa, One of the Riu Kiu Islands" (1912), lists a species, *Aëtobatis tobijei,* new to the present writer, but gives no data whatever about it. But Jordan and Fowler (1903), in their "Review of the Elasmobranchiate Fishes of Japan," call this same ray *Myliobatis tobijei* and note that its teeth are pavement-like in several series in each jaw. Furthermore Bleeker (1854), the original describer of this ray, names it *M. tobijei* and says "Upper jaw broader than long with laminated teeth; lower longer than wide, with median hexagonal teeth varying from more than twice to more than three times broader than wide." From this we see that Snyder's fish is not an *Aëtobatus* at all, but a *Myliobatis.*

SYNONYMY.

SYNONYMY OF THE SPOTTED EAGLE RAY, AËTOBATUS NARINARI, IN CHRONOLOGICAL ORDER.

1614. *Narinnary.* Abbeville, Hist. Mission Pères Capucins Isle Maragnan, p. 245, Brazil.

1633. *Narrinnari.* Laet, Novus Orbis, Seu Descript. Indiæ Occid., p. 616, Brazil.

——. *Narinari.* Johann Moritz von Nassau Siegen, Brazilianisch Naturgegenstände, I, Tafel 332, Brazil.

——. *Narinari.* Theatri Rerum Naturalium Brasiliæ, Tome I, Icones Aquatilium, fig. 31, Brazil.

1648. *Narinari.* Marcgrave, Historia Rerum Naturalium Brasiliæ, pp. 175–176, figure, Brazil.

1648. *Narinari.* Piso, De Medicina Brasiliensi, p. 44, Brazil.

1658. *Narinari.* Piso, Historia Naturalis & Medica Indiæ Occidentalis, pp. 58 and 293, figures, Brazil.

1686. *Narinari.* Willughby, De Historia Piscium, p. 66, tab. C, fig. 5.

1697. *Pastinaca marina.* Sloane, Phil. Trans., vol. XIX, pp. 674–676, figure, teeth, Jamaica.

1713. *Narinari.* Ray, Synopsis Methodica Avium & Piscium, p. 24.

1718. *Narinari.* Ruysch, Theatrum Universale Omnium Animalium, Piscium, etc., p. 146, tab. XXXIX, fig. 6.

1725. *Whip Ray.* Sloane, Natural History of Jamaica, pp. 276–277, Jamaica.

1756. *Whip Ray.* Browne, Civil and Natural History of Jamaica, p. 459, Jamaica.

1767. *Narinari.* Jonston, Historia Naturalis de Piscibus et Cetis, pp. 208–209, tab. XXXIX, fig. 6.

1790. *Raja narinari.* Euphrasen, Kong. Svens. Vet. Nya Handl., XI, pp. 217–219, tab. X. First binomial name, species established. St. Bartholomew, West Indies.

1792. *Raja narinari.* Walbaum, Artedi's Bibliotheca Ichthyologica, III, p. 528.

1801. *Raja narinari.* Bloch and Schneider, Systema Ichthyologiæ, p. 361.

1803. *Raja ocellata.* Russell, Fishes of Coromandel, p. 5, pl. VIII, Coromandel.

1816. *Aëtobatus narinari.* Blainville, Journal de Phys., de Chem., et d'Hist. Nat., LXXXIII, pp. 261–262. Generic name first established.

1817. *Raja narinari.* Cuvier, Le Regne Animal, t. II, p. 138.

1830. *Myliobatis narinari.* Bennett, Memoir Life of Raffles, p. 694. East Indies.

1835. *Myliobatis eeltenkee.* Ruppell, Fische des Rothen Meeres, Neue Wirbelthiere, Fauna Abyssinien, pp. 70–71, fig. teeth, Red Sea.

1836. *Raja narinari.*
Aëtobatis narinari.
Myliobatis narinari.
Agassiz, Poissons Fossiles, III, pp. 79–80 and 325, tab. D, figs. 1 and 2; tab. 46, figs. 4 and 5 (all teeth).

1839. *Aëtobatis indica.* Swainson, Nat. Hist. Fish. II, p. 321.

1841. *Aëtobatis narinari.* Müller and Henle, Systematische Beschreibung Plagiostomen, pp. 179–181. The form first definitely established under full diagnosis of characters.

1817. *Myliobatis narinari.* Cuvier, Le Regne Animal, vol. Poissons, pl. 118, No. 4*a* (teeth).

1844. *Raja edentula.* Forster (Lichtenstein ed.) Descriptiones Animalium, etc., pp. 227–229, South Seas.

1840 to 1845. *Aëtobatis narinari.* Owen, Odontography, pp. 46–48, pl. 16, fig. 1 (section through dried head).

1849. *Stoasodon narinari.* Cantor, Jour. Asiatic Soc., Bengal, XVIII, pp. 434–435, Bay Bengal.

1852. *Aëtobatis narinari.* Bleeker, Verh. Bataav. Gen. Kunst. en Weten., XXIV, p. 88, East Indies.

1859. *Goniobatis meleagris.* Agassiz, Proc. Bost. Soc. Nat. Hist., VI, p. 385, Sandwich Islands.

1861. *Aëtobatis narinari.* Duméril, Arch. Mus. Hist. Nat., x, pp. 241–243, pl. 20, fig. 2 (head).
1865. *Aëtobatis narinari.* Duméril, Hist. Nat. Poissons, i, p. 640.
1865. *Aëtobatis narinari.* Day, Fishes of Malabar, pp. 280–281, Indian Ocean.
1867. *Aëtobatis laticeps.* Gill, Ann. Lyc. Nat. Hist. New York, viii, p. 137.
1870. *Aëtobatis narinari.* Günther, Cat. Fishes Brit. Mus., viii, pp. 492–493.
1871. *Aëtobatis narinari.* Klunzinger, Verh. K. K. Zool-Botan. Gesell. Wien, xxi, pp. 685–686, Red Sea.
1877. *Aëtobatis narinari.* Yarrow, Proc. Acad. Nat. Sci. Phila., xxix, p. 216. Beaufort.
1878. *Aëtobatis narinari.* Day, Fishes of India, ii, p. 743, fig. 4, pl. cxciv. Indian Ocean.
1879. *Aëtobatis narinari.* Jordan and Gilbert, Proc. U. S. Nat. Mus., i, p. 386, Beaufort.
1880. *Aëtobatis narinari.* Günther, Introd. Study Fishes, pp. 344–345, fig. 130.
1881. *Aëtobatus narinari.* Poey, Fauna Puerto-Riqueña, p. 349.
1882. *Aëtobatis narinari.* Jordan and Gilbert, Synopsis Fishes North America, p. 50.
1883. *Aëtobatus narinari.* Stahl, Fauna de Puerto Rico, pp. 81 and 167.
1886. *Aëtobatis narinari.* Douglas-Ogilby, Proc. Linn. Soc., New South Wales, x, p. 466, New South Wales.
1886. *Aëtobatis narinari.* Günther, Encyc. Britan., xx, p. 300, fig. 3.
1887. *Stoasodon narinari.* Jordan, Proc. U. S. Nat. Mus., ix for 1886, pp. 26 and 558, Beaufort and West Indies.
1895. *Stoasodon narinari.* Henshall, Bull. U. S. Fish Comm., xiv, p. 210, W. Coast Florida,
1895. *Aëtobatus narinari.* Jordan, Proc. Calif. Acad. Sci., ser. 2, v, p. 381, West Coast of Mexico.
1896 *Aëtobatus narinari.* Jordan and Evermann, Fishes North and Middle America.
1900 i, 88; iv, pls. xv and xvi, figs. 37 and 37*a*.
1896 *Aëtobatus laticeps.* Jordan and Evermann, Fishes North and Middle America, i,
1898 p. 88–89; iii, p. 2753.
1900. *Aëtobatus narinari.* Evermann and Marsh, Bull. U. S. Fish Comm., xx, pt. i, p. 67. Porto Rico.
1901. *Aëtobatis narinari.* Steindachner, Denk. Kais. Akad. Wiss., Wien, lxx, p. 517, Laysan.
1904. *Aëtobatus narinari.* Jenkins, Bull. U. S. Fish Comm., xxii, p. 421, Honolulu.
1905. *Stoasodon narinari.* Jordan and Evermann, Bull. U. S. Fish Comm., xxiii, pt. i, pp. 49–50, Hawaii.
1905. *Stoasodon narinari.* Jordan and Thompson, Bull. U. S. Bureau Fisheries, xxiv, p. 232, Tortugas.
1905. *Aëtobatus narinari.* Jordan, Guide to Study of Fishes, i, p. 557.
1907. *Aëtobatus narinari.* Smith, Fishes of North Carolina, p. 46, Beaufort.
1907. *Stoasodon narinari.* Jordan and Seale, Bull. U. S. Bureau Fisheries, xxvi, p. 4, Cavite, P. I.
1907. *Aëtobatus narinari.* Miranda Ribeiro, Arch. Mus. Nac. Rio Janeiro, xiv, Coast of Brazil.
1910. *Aëtobatis narinari.* Günther, Jour. Mus. Godeffroy, xvii, pp. 496–497, Samoa.
1910. *Aëtobatus narinari.* Coles, Bull. Am. Mus. Nat. Hist., xxviii, pp. 338–341, Cape Lookout.
1910. *Aëtobatus narinari.* Gudger, Am. Nat., xliv, pp. 399–400, Beaufort.
1910. *Aëtobatis narinari.* Annandale, Mem. Ind. Mus., pp. 4–5, pl. ii, fig. 2 (young), Bay of Bengal.
1912. *Aëtobatus narinari.* Gudger, Proc. Biol. Soc. Wash., xxv, pp. 150–152.
1913. *Aëtobatus narinari.* Gudger, Proc. Biol. Soc., Wash., xxvi, p. 101.
1913. *Aëtobatus narinari.* Coles, Bull. Am. Mus. Nat. Hist., xxxii, pp. 30–33, figs. 1–2; pl. iii, figs. 1, 2, 3.

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1859. Goniobatis, a new species of skate from the Sandwich Islands. Proc. Boston Soc. Nat. Hist., vol. vi, p. 385, 1856–1859.

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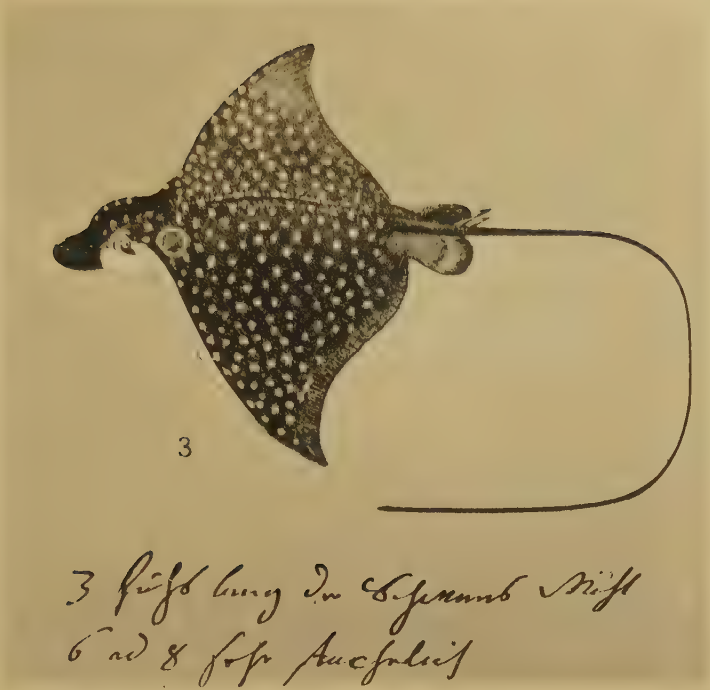
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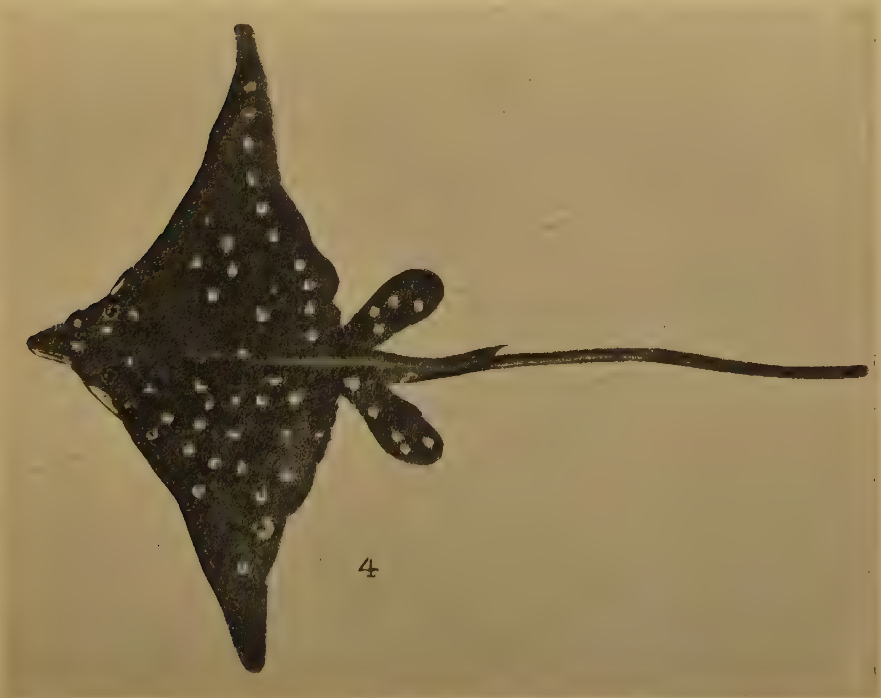
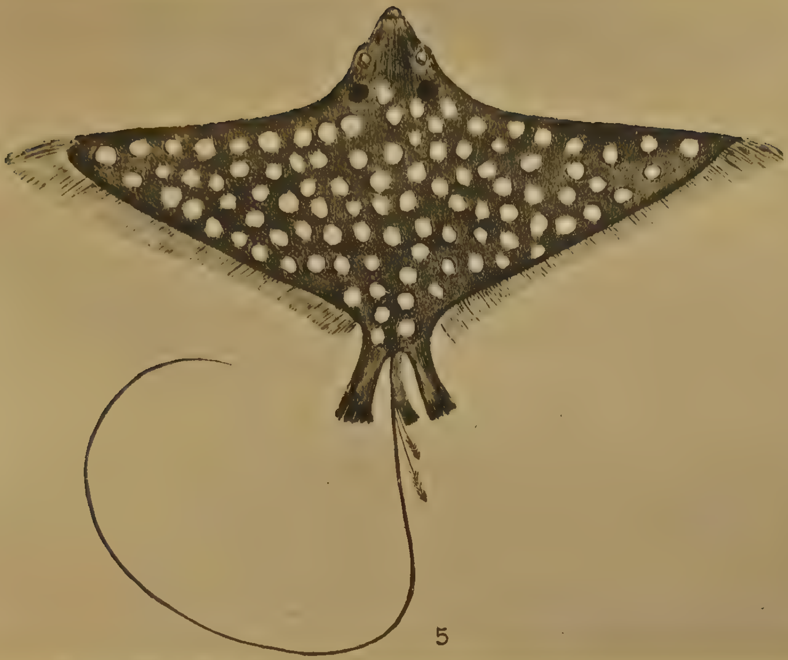


FIG. 3. *Narinari*, after Marcgrave's water-color painting in the Royal Library of Berlin. Brazil, 1648.

FIG. 4. *Narinari*, after Poste's oil painting in the Royal Library of Berlin. Brazil 1648.



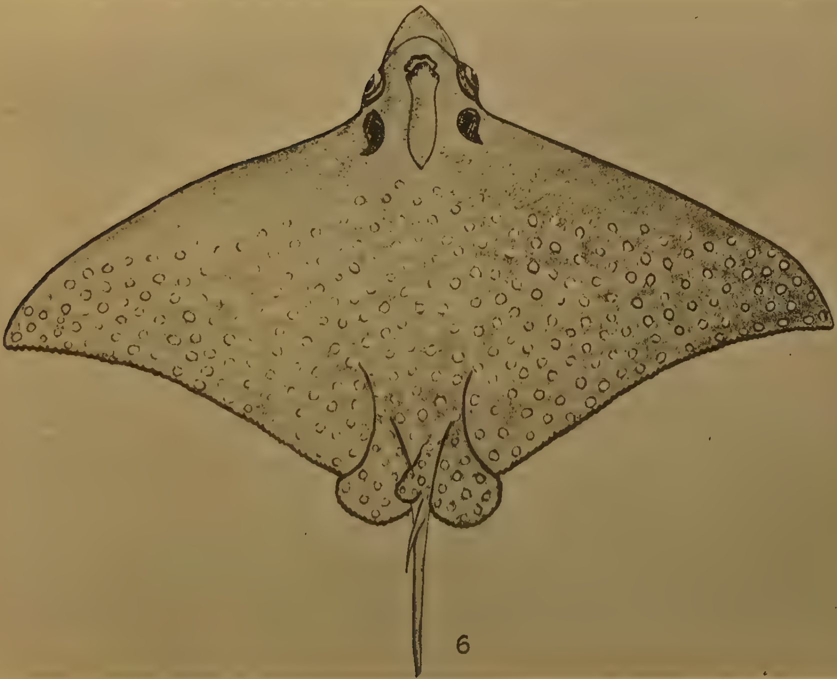
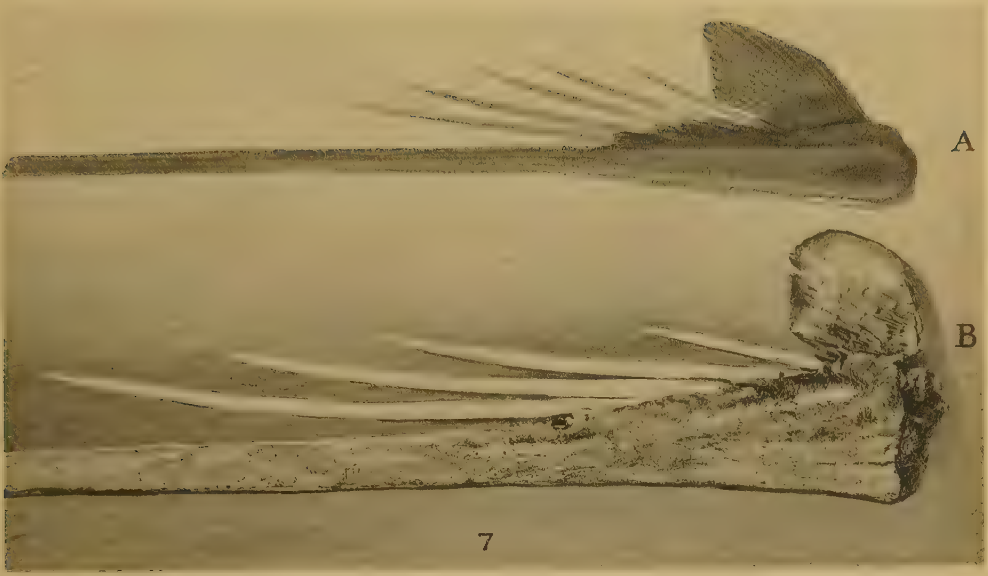


FIG. 5. *Raja narinari*, after Euphrasen, 1790, West Indies.
FIG. 6. *Raja ocellata*, after Russell, 1803, Coromandel.



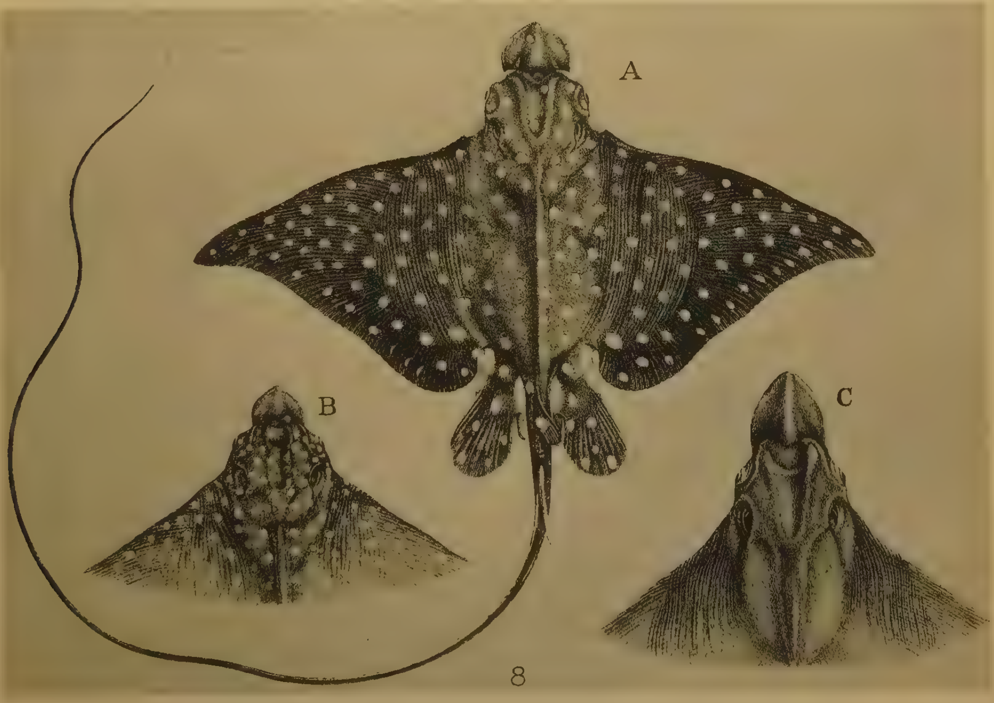
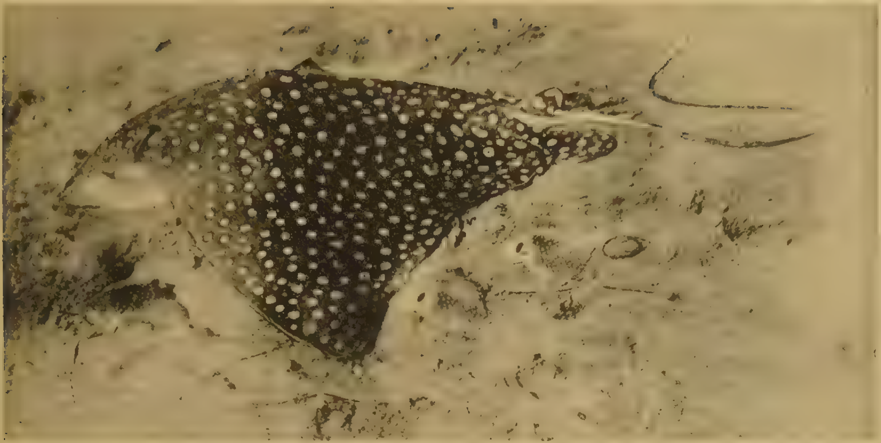


FIG. 7. A. Tail of *Raja quinqueaculata*, after Quoy and Gaimard, 1824, Guam. B. Tail of *A. narinari*, Beaufort.

FIG. 8. A. *A. latirostris;* B. *A. narinari;* C. *A. fouet* (*A. flagellum?*) after A. Dumiril, 1865.



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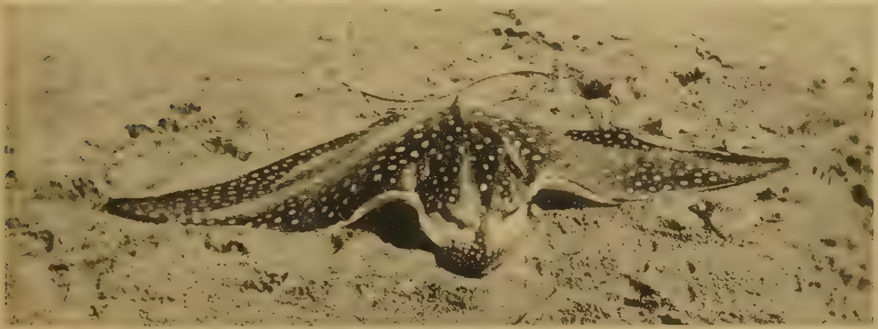

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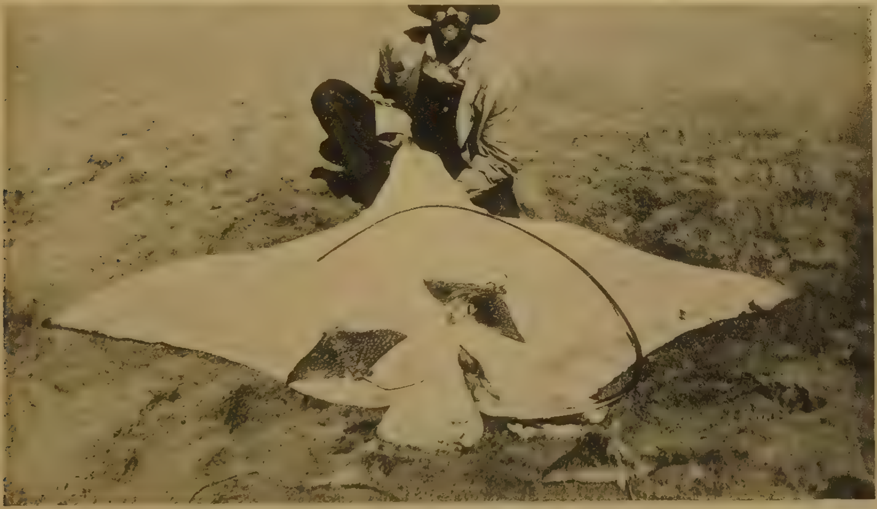
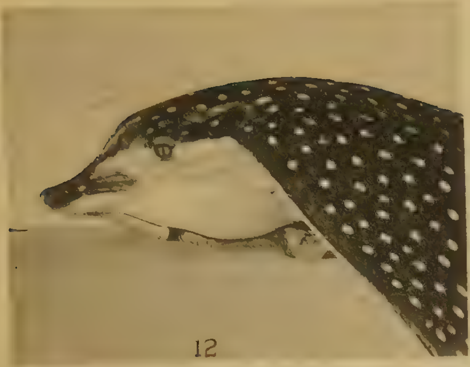


11

Aetobatus narinari and Embryos. After Coles 1913.

A. narinari (Euphrasen); female, 7 ft. 2 in. in diameter, and the four embryos to which she gave birth at intervals of a few seconds, on the beach. Cape Lookout, N. Carolina.

FIG. 9. Seen from the side. FIG. 10. From in front. FIG. 11. From below. In figure 9 note unusual coloration consisting of ocelli instead of white spots.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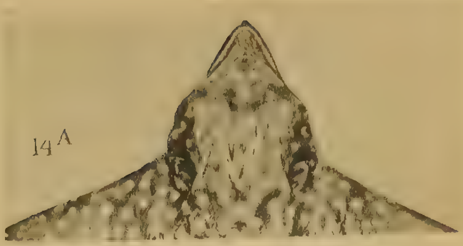
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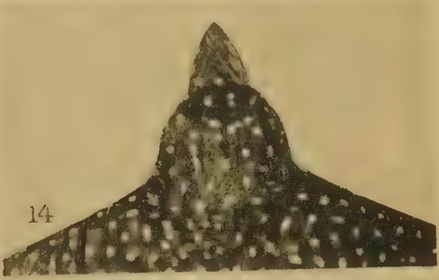
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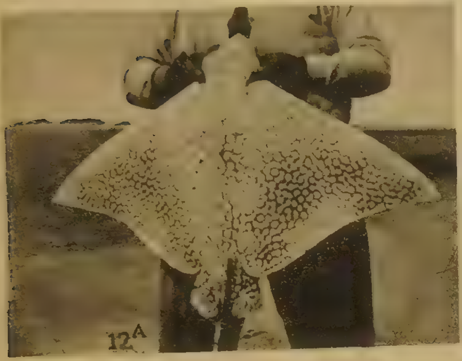
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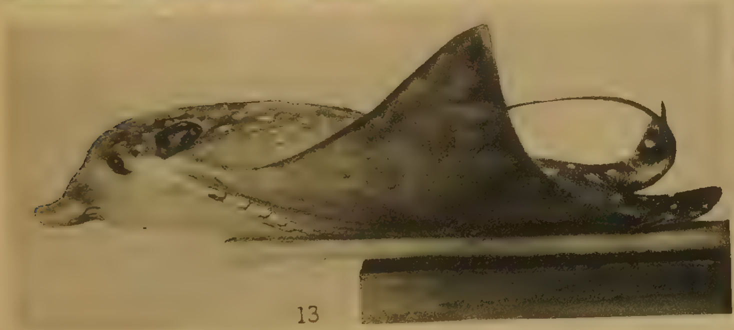
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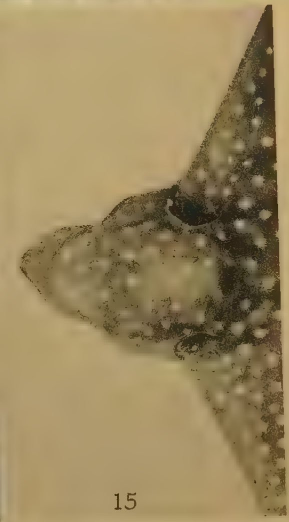
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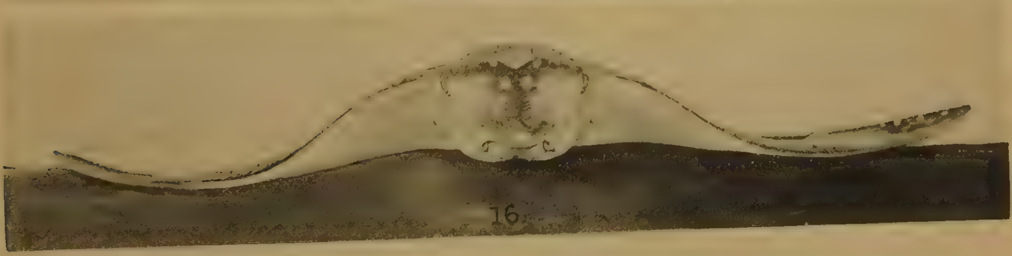
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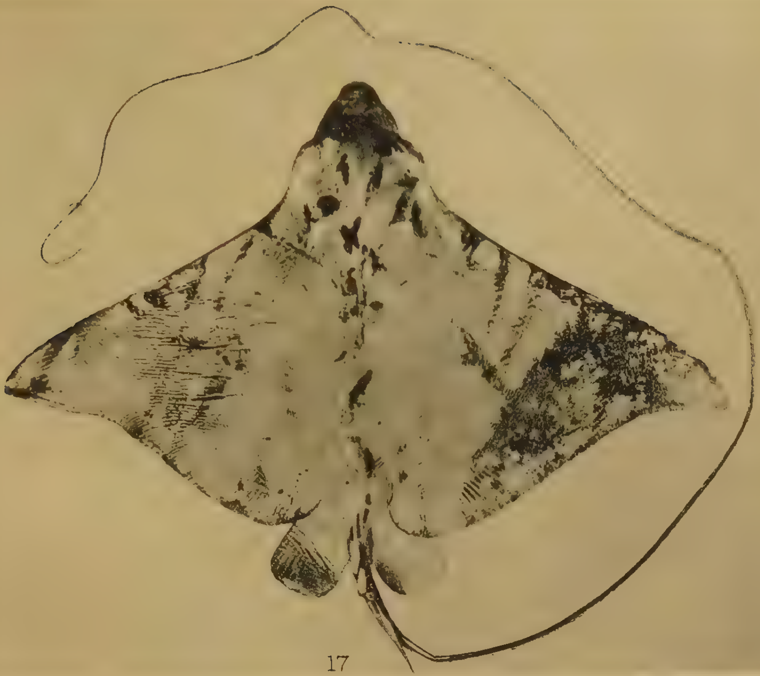
FIG. 12. Lateral view of head of *A. narinari*, Beaufort, 1910 (fresh specimen).
12A. *A. narinari*, an albino female taken at Cape Lookout, 1913.
FIG. 13. Lateral view of *A. narinari*, Beaufort, 1909 (formalin specimen).
FIG. 14 and 14A. Heads of *A. narinari*, Beaufort, 1910 (fresh specimen).
FIG. 15. Head of specimen shown in figure 13.
FIG. 16. Front view of Spotted Eagle Ray, Beaufort, 1910 (fresh specimen)



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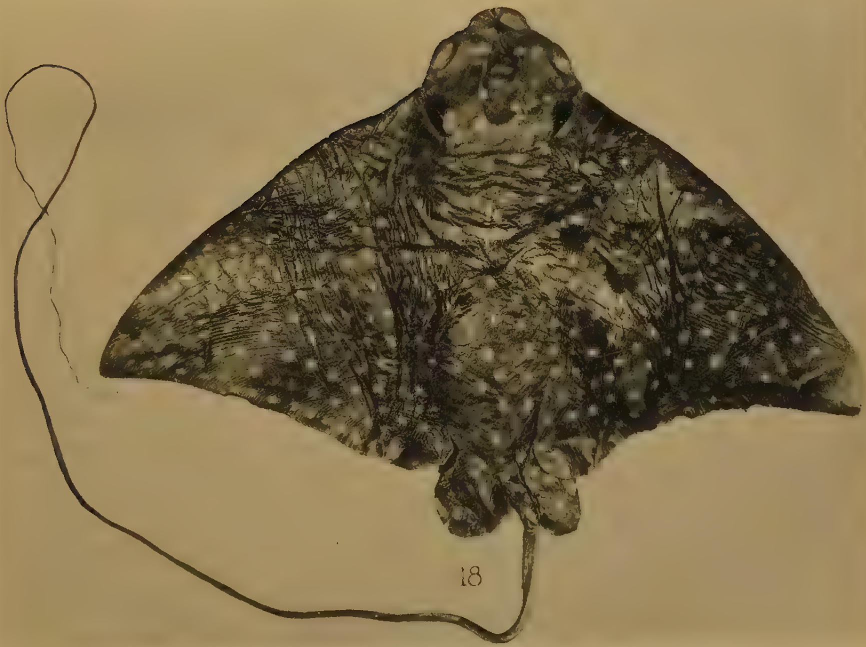

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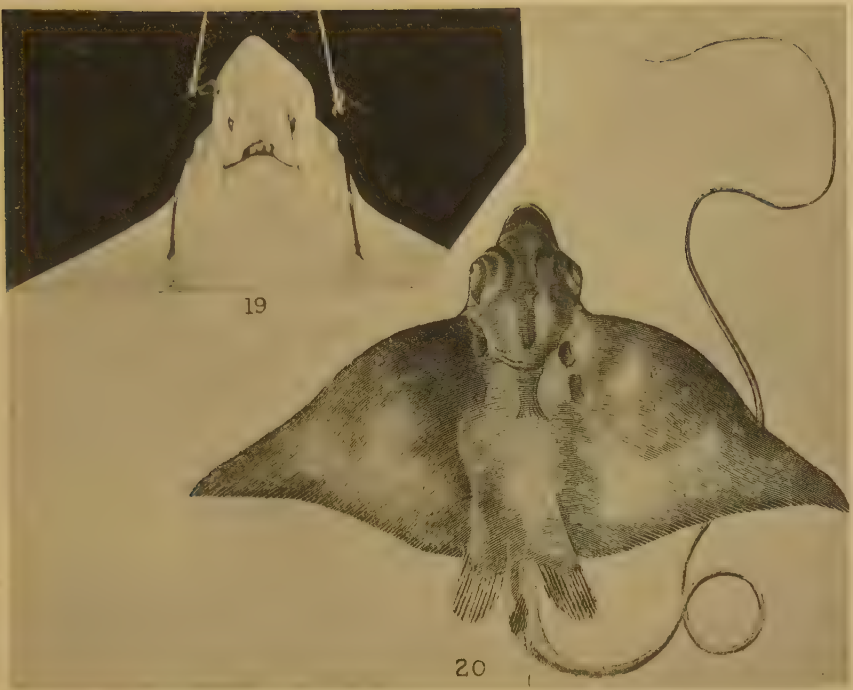
Fig. 17. *A. narinari*, young, after Annandale, 1910, Bay of Bengal
Fig. 18. Embryo of Spotted Eagle Ray. One of the four obtained from the ray shown in Plate V.



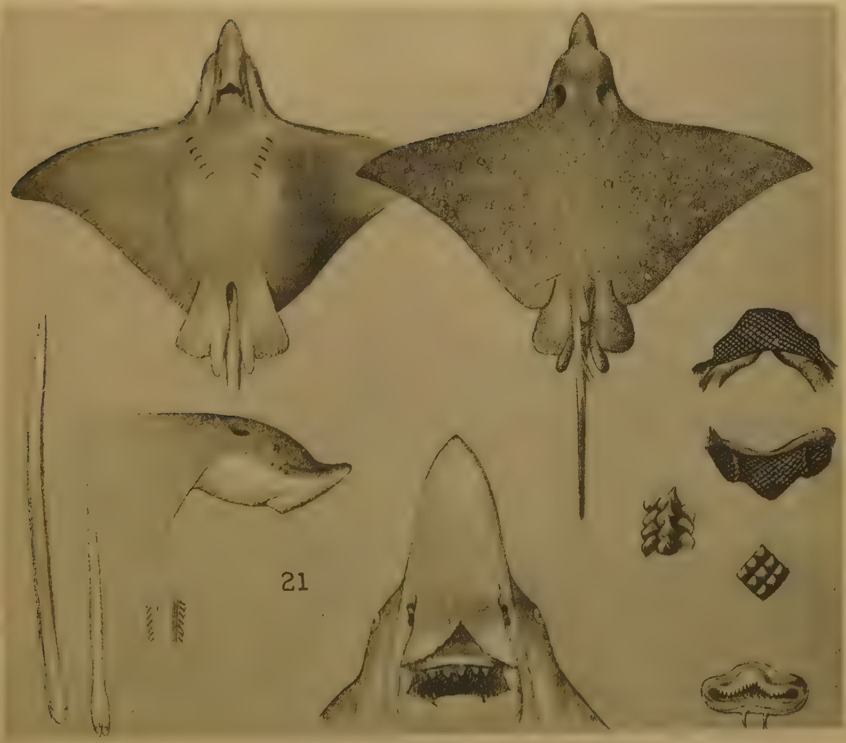
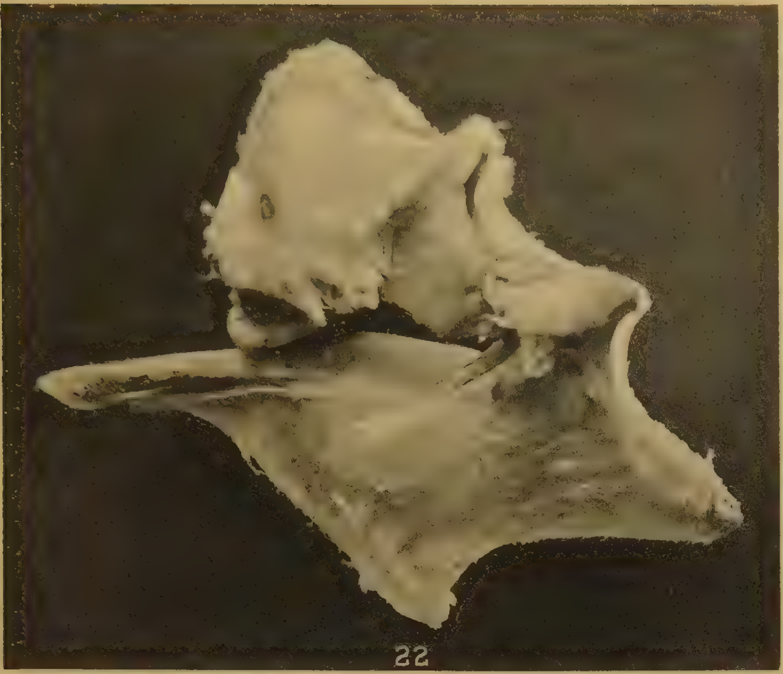


FIG. 19. Ventral view of head of ray shown in figures 13 and 15, plate VI.

FIG. 20. *A. guttata*, after Shaw-Lacepede-Commerson.

FIG. 21. *Myliobatis punctatus*, after Miklouho-Maclay and Macleay, 1886, Hermit Islands.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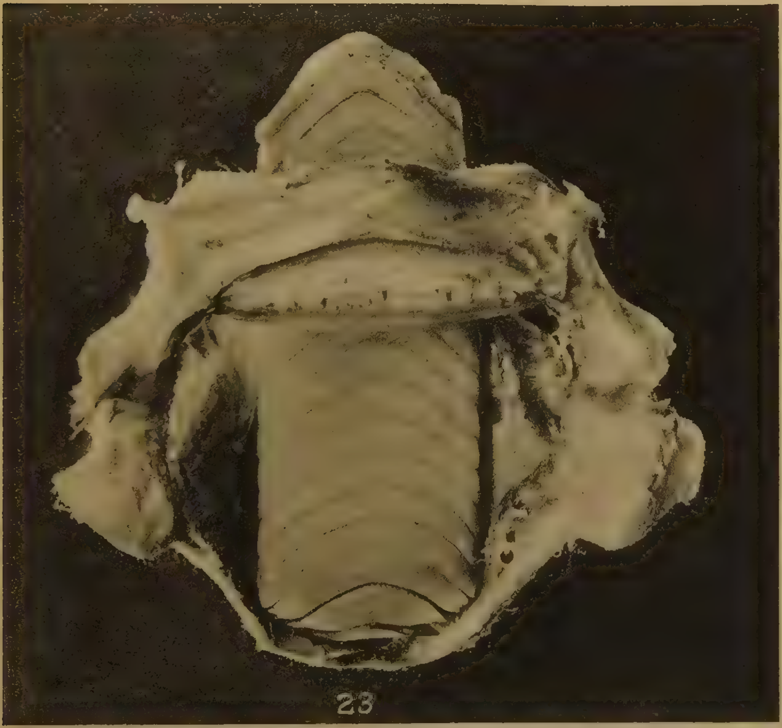
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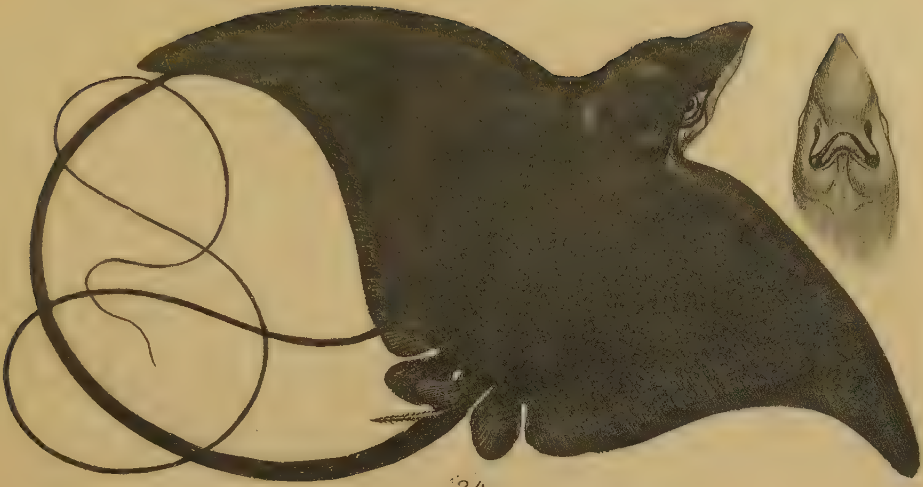
FIG. 22. Side view of jaws of *A. narinari* from Beaufort.
FIG. 23. Jaws of *A. narinari* seen from above.



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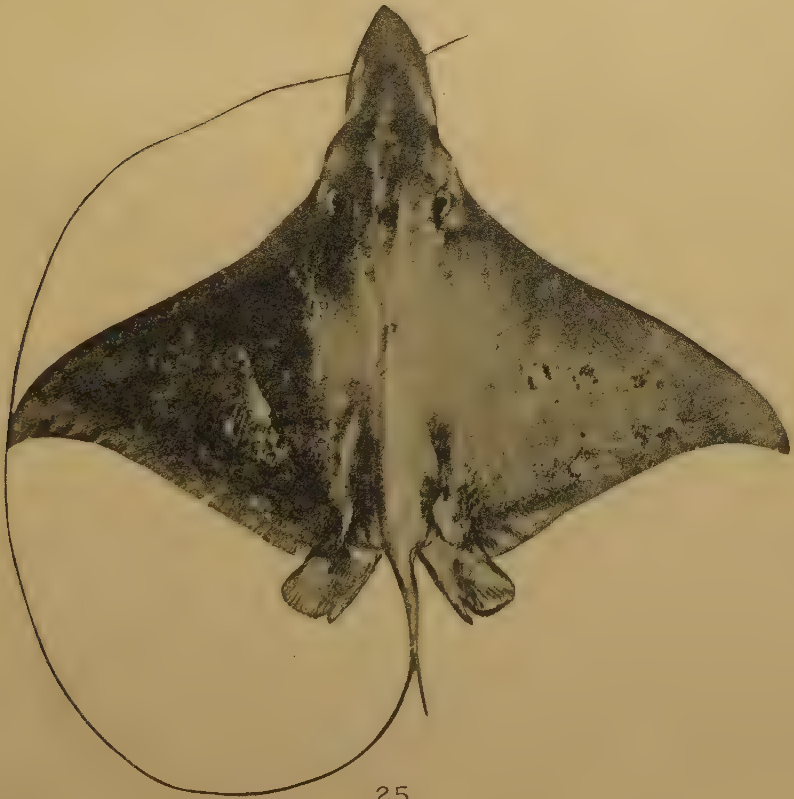

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FIG. 24. *Raja flagellum*, after Bloch and Schneider, 1801.
FIG. 25. *Aetobatus flagellum*, after Annandale, 1910, Bay of Bengal.